United States
Department of
Agriculture

**Agricultural
Research
Service**

ARS-71 (1993)

May 1993

Beef Research

Progress Report No. 4

Roman L. Hruska U.S. Meat Animal Research Center
in cooperation with the
University of Nebraska College of Agriculture,
Agricultural Experiment Station

U.S. Department of Agriculture. 1993. Beef Research Progress Report No. 4. U.S. Department of Agriculture, Agricultural Research Service, ARS 71 (1993), 187 pp.

This report represents a cross section of our cattle research program at the present time. The report includes research results in genetics, meat quality, meat safety assurance, animal health, reproduction, nutrition, and production systems. Since some of the projects from which results are reported are still in progress, the preliminary nature of some of the results must be recognized. However, it is our opinion that information useful to the industry should be provided at the earliest possible time. Progress reports of this nature will be released periodically to make current results available to the beef cattle industry.

Keywords: Beef cattle, breeds, meat safety, genetics, meat quality, production, meat safety, nutrition, stress.

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U.S. Meat Animal Research Center
Agricultural Research Service
United States Department of Agriculture
P.O. Box 166
Clay Center, Nebraska 68933

Library of Congress ISSN 0193-3787

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ANIMAL HEALTH SYSTEMS

ROMAN L. HRUSKA
U.S. MEAT ANIMAL RESEARCH CENTER[1]

The Roman L. Hruska U.S. Meat Animal Research Center (MARC) is part of the U.S. Department of Agriculture's Agricultural Research Service. MARC was authorized by Congress on June 16, 1964, thereby creating a single facility that provides an unusual opportunity for making contributions to the solution of problems facing the U.S. livestock industry. The 35,000-acre facility and the office-laboratory buildings provide a physical plant for about 80 scientists and about 200 support personnel. In addition, the University of Nebraska's Great Plains Veterinary Educational Center (GPVEC) provides a facility for four university faculty members, support personnel, and pre- and post-DVM students.

Approximately 50 percent of the research program is devoted to beef cattle, 30 percent to swine, and 20 percent to sheep. Current research program objectives require breeding age female populations of approximately 7,250 cattle (20 breeds), 4,250 sheep (10 breeds), and 700 crossbred swine litters to carry out the various experiments.

The research program at the Center is organized on a multidisciplinary team basis to solve national problems for the U.S. livestock industries. Some projects are focused on the specific research needs of USDA Action Agencies. The seven research units are Genetics and Breeding, Nutrition, Reproduction, Meats, Animal Health Systems, Biological Engineering, and Production Systems. The research program complements research conducted elsewhere by the U.S. Department of Agriculture and is cooperative with the University of Nebraska Institute of Agriculture and Natural Resources and other land grant university agricultural experiment stations throughout the country.

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[1]Agricultural Research Service-U.S. Department of Agriculture, the University of Nebraska, and other cooperating land grant universities.

MARC's beef cattle research program places the highest priority on developing technology capable of having an immediate and long-term impact on the beef cattle industry. The program includes research and development of technology that can be implemented by small farmers, large commercial producers, and agri-business.

Currently, we have 65 research scientist and research associate positions at MARC.

Appreciation: I want to express appreciation to the scientists for their contributions, to Ralston Graham for his editorial comments, and to Kris Schrick, Public Affairs Specialist, for organizing and editing this report.

D.B. Laster, Center Director
Roman L. Hruska
U.S. Meat Animal Research Center

Beef Facilities and Management at MARC

W. Gordon Hays and Gary S. Ross[1]

The Cattle Operations Unit functions as a support service to the research scientists and maintains the animal populations necessary for our livestock research. Indirectly, this also involves responsible land management and herd health procedures. All the facilities and procedures employed in maintaining the extensive cattle herd are determined by research needs. Consequently, while providing a function sometimes indirectly related to research, the operations unit is necessary to provide adequate feedstuffs and healthy animals for research studies.

Cattle Management

The cow herd of 7,300 breeding age females are managed so that 80% of the cows and heifers will calve during the spring season (March through May) and 20% will calve during the fall season (August through early October).

Yearling heifers are bred two to three weeks before the mature cow herd. About half of the heifers and cows are bred naturally and the other half artificially inseminated (A.I.). The A.I. seasons vary from 25 to 45 days depending on experimental requirements. All cows are exposed to natural service sires following the A.I. season.

In many populations all heifers are retained for breeding. Because of the high heifer retention rate a very young cow herd is maintained. Also many prime age pregnant cows become excess to the research needs each year. These females as well as a limited number of bulls are sold in two production sales. The first auction sale is held in late January. Pregnant spring calving cows and mature herd bulls are sold at that time. An early May sale merchandizes excess fall calving cows and yearling bulls.

Facilities

Cow-Calf Polesheds. Nine polesheds at MARC are used for maintenance of the breeding herd. Each barn functions as a working area, with general-purpose facilities designed for calving, artificial insemination, pregnancy checking, data collection, and routine processing of the cattle herd. These facilities generally include a scale, squeeze chute, calf-pulling stall, and individual pens (ranging from 10 to 25, depending upon use in cow or heifer calving areas). Individual pens are used primarily in the spring during the main calving season and are used either after assistance to the cow or heifer during calving or to provide assistance to the calf in cases of severe chilling, poor mothering, or sickness. Corrals are used to hold or sort cattle. Each area is equipped with a "hot house," which is a heated office and supply area.

Bull Barn. This area is used for routine processing, semen collection, and special research studies. Pens are available for holding and sorting bulls. A heavily constructed squeeze alley and chute are used for processing and semen collection. The hot house includes an office and lab for semen evaluation.

Feedlot. Over 5,000 calves and assorted other cattle are fed in the feedlot, primarily in the winter. This number includes animals which will be used in the breeding herd, animals fed for slaughter and cows for reproduction studies. Performance and puberty studies are routinely conducted on many of the young calves as part of genetics studies. Approximately 80% of the calves are born in the spring and come to the feedlot in the fall at an average age of six months. Twenty percent of the calves from the fall calving herd enter the feedlot at approximately five months of age.

Multipurpose Building. The main processing facility is a pre-engineered metal building, fully lighted and heated, with concrete flooring. The working facility includes a circular squeeze, working alley, scale, and chute. Fifteen pens are used for sorting and holding. There is also an office and lab area. A reproductive physiology lab is a separate, thermally controlled area specifically designed for embryo transfer and other cattle physiology research.

Scalehouse. This is a metal building which functions as the main treatment area and as office headquarters for the feed-truck drivers. A working alley, scale, and chute are included in this area, as well as sorting pens and sick pens.

Poleshed. This barn functions as a sale facility. There is a heated office and sale ring.

Cattle Confinement Area. There are 11 pre-engineered metal buildings with a total animal capacity of 1,500 head. They are used mainly for intensive nutrition, reproduction, or environmental research.

The cattle surgery facility includes a prep room, surgery room, recovery stalls, lab, and office. Four barns are equipped with individual headgates for intensive feeding studies. Two of these accommodate cows with calves and have been used predominantly for cow efficiency studies. The other two are used for postweaning experiments requiring individual feed consumption data.

A specially designed barn includes 12 metabolism crates, used to study animal utilization of nutrients. In addition, 36 stalls equipped with headgates are primarily used for studies requiring frequent collection of blood samples for hormonal determinations. Three hood calorimeters are used for fasting heat production studies. A nursery has been developed for artificial rearing of calves for specific research studies. The barn also contains a laboratory.

Two self-cleaning buildings are equipped with flushing gutters and are used for total confinement research. Working facilities include an office, lab, crowding area, working alley, scale, chute, and sorting pens.

Land Management

The land is managed so that 27,000 acres of cool and warm-season grasses are used as pastures. Twenty-five thousand acres are used for the cattle herd. Cows are maintained on pastures year-round and supplemented with hay and/or silage in the winter. Heifers are supplemented with a haylage-corn silage diet through their first calving. Bulls are managed similarly to the cows.

Six thousand acres of land are irrigated for crops and hay production. The two main feedstuffs produced at MARC are alfalfa and corn silage. The first cutting of alfalfa is chopped for haylage and subsequent cuttings are harvested for hay. Corn acreage produces 50,000 tons of silage. (All feedstuffs are used for both the sheep flock and the beef herd. Corn is also a major component of the swine diet.) Additional acreage includes irrigated pasture and small grains used for forage and feed.

[1]Hays is the cattle operations manager, and Ross is the herd health veterinarian, MARC.

Herd Health Procedures

The herd health veterinarians and staff work closely with cattle operations personnel to assist in the efficient managing of these areas. The University of Nebraska Great Plains Veterinary Education Center faculty and Kansas State University College of Veterinary Medicine students work closely with the MARC herd health veterinarians to provide care for the research populations.

Quarantine of purchased animals and testing for evidence of disease; monitoring the causes of sickness and death; and the monitoring of blood antibodies to various important diseases are procedures used to prevent disease and to determine which diseases need to be controlled by vaccination programs. The following are the vaccination and routine processing procedures for heifers, cows, calves, and bulls.

Heifers: Prior to their first breeding season, yearling heifers are injected with modified live virus (MLV) IBR-BVD (infectious bovine rhinotracheitis - bovine viral diarrhea), 5-way leptospira/vibrio in oil, and 7-way clostridial vaccines. Approximately 70 days after the end of the breeding season, heifers are palpated for pregnancy, injected with ivermectin for parasite control, and vaccinated against *E. coli* bacteria. Prior to calving, brands are clipped, and the heifers are given a booster *E. coli* and 7-way clostridial vaccine and a Vitamin A and D injection.

Cows: After calving and before breeding the cows are given the same vaccines as the heifers (except the MLV IBR-BVD which is given to the cows on even numbered years). At 70 days postbreeding, they are pregnancy checked and treated for external and internal parasites (ivermectin). Prior to calving they receive the same booster vaccinations and vitamin injections as the heifers. Fly control among breeding stock is achieved by the use of insect impregnated ear tags and periodic group spraying with appropriate insecticides. Cows become excess for research if they fail to conceive or are no longer needed for their projects.

Calves from Birth to Maturity: At birth, all calves are identified, weighed, dehorned (caustic paste), the navel disinfected with iodine, and vaccinated against viral scours. Depending on the research project, some bull calves may be castrated. Prior to the cow breeding season, the calves are vaccinated with 7-way clostridium and 5-way leptospira vaccines. Approximately three weeks prior to weaning, they receive a second dose of clostridial and leptospira vaccines along with (MLV) IBR-BVD. At weaning no vaccines are given, but the calves are weighed and sorted to group pens in the feedlot. One month postweaning the calves receive a booster (MLV) IBR-BVD vaccination and are given ivermectin for parasite control. Strain 19 brucella vaccine is given to heifers at approximately 8-9 months of age. A majority of the bulls are castrated following weaning with a bloodless banding technique. At one year of age some of the bulls are sold as breeding stock, and the rest of the heifers, bulls, and steers are either used in research studies or are fed out for slaughter. Standard disease identification signs and treatment procedures are used in the cow-calf areas and at the feedlot for all calves to ensure uniformity of treatment and compliance with accepted meat quality assurance programs.

Bulls: At the end of the growing period (1 yr), bulls are vaccinated with (MLV) IBR-BVD, 7-way clostridium, and 5-way leptospira. Subsequently, they are treated for external parasites in the fall, and vaccinated with (MLV) IBR-BVD on even numbered years prior to breeding season. Breeding soundness examinations are conducted on all bulls prior to the breeding season.

Each fall all cows and heifers 24 months of age and older and all bulls nine months and older are tested for brucellosis. This has been done since 1988 and the MARC cattle herd is Certified Brucellosis Free.

Cycle V of the Germplasm Evaluation (GPE) Program in Beef Cattle

Larry V. Cundiff, Keith E. Gregory, and Robert M. Koch[1]

Introduction

Results from the first four cycles of the Germplasm Evaluation (GPE) Program at MARC demonstrated that vast genetic variation exits among and within breeds for most bioeconomic traits in beef cattle. The range for differences between breeds was comparable in magnitude to the range in breeding value of individuals within breeds for most traits. Thus, significant genetic change can result from selection both between and within breeds. Breeds can be selected to optimize performance levels for important bioeconomic traits with a high level of precision much more quickly than intrapopulation selection.

No single breed or biological type excels in all traits important in beef production. Breeds with the greatest retail product growth potential excel in gain per unit feed consumed to wt and time endpoints; however, they also 1) sire progeny with heavier birth wt and increased calving difficulty; 2) produce carcasses with lower levels of marbling; 3) tend to be older at puberty if milk production has not been emphasized in their selection history; and 4) have heavier mature wt increasing nutrient requirements for maintenance. Use of crossbreeding systems that exploit complementarity by terminal crossing of sire breeds noted for lean tissue growth efficiency with crossbred cows with optimum size, milk production and adaptation to the feed and climatic environment provide the most effective means of managing these trade-offs.

Results from Cycle IV of the GPE program demonstrated that breeds that have been selected for muscular hyperplasia (i.e., double muscling) may be an appropriate choice as a terminal sire breed to produce progeny (all steers and heifers would be slaughtered) suited for markets targeting lean-low caloric beef products. Piedmontese crosses ranked eighth (comparable to original Hereford Angus crosses) among the 11 breed groups in final wt, but ranked second to Charolais in wt of totally trimmed (0 mm) retail product due to exceptional dressing percentage and significantly higher retail product percentages than other breeds. Marbling was low in Piedmontese crosses, but estimates of meat tenderness were relatively high. Belgian Blue, another breed that has been selected for muscular hyperplasia, will be evaluated in Cycle V of the GPE program.

Bos indicus X *Bos taurus* crosses were exceptionally productive and efficient cows. A cooperative experiment conducted jointly at MARC and the Subtropical Agricultural Research Station, Brooksville, FL revealed that advantages of *Bos indicus* X *Bos taurus* cross cows over *Bos taurus* X *Bos taurus* cross cows were even greater under subtropical climatic conditions than under temperate climatic conditions. However, the advantages of *Bos indicus* crosses were tempered by increased birth wt and calving difficulty when *Bos indicus* bulls were mated to *Bos taurus* females, older age at puberty, increased mortality when born in colder seasons in temperate areas and reduced meat tenderness as the proportion of *Bos indicus* inheritance increased. In Cycle V, tropically adapted breeds that have evolved separately for at least 2,000 yr from European *Bos taurus* breeds and Asian *Bos indicus* breeds will be sampled and evaluated. Specific objectives are to determine whether the earlier age at puberty and increased meat tenderness characteristic of *Bos taurus* germplasmhas been retained or has shifted to levels characteristic of *Bos indicus* germplasm in response to selection for adaptation to tropical conditions.

Procedure

The GPE program has been conducted in five cycles. Table 1 shows the mating plan for each cycle. In Cycle V, as in previous cycles of the program, the base cows include Angus (about 500) and Hereford (about 350) cows calving at 4 yr of age or older. In addition, about 550 composite MARC III (1/4 Angus, 1/4 Hereford, 1/4 Pinzgauer and 1/4 Red Poll) cows calving at 4 yr of age or older are included in Cycle V. The cows are being mated to produce F_1 crosses by the following sire breeds.

Hereford and Angus. Semen from polled and horned Hereford bulls and from Angus bulls is being used to produce F_1 cross progeny. Hereford-Angus reciprocal crosses have been used as a reference breed throughout the GPE program to facilitate pooling of data and comparison of breeds in different cycles. More than 30 bulls of each breed, some of which were included in Cycle IV (born from 1982-1984) and others born since 1988, are being used in Cycle V.

Tuli. The Tuli, a Sanga type of cattle (non humped), are believed to trace to crosses between original humpless longhorn types (*Bos taurus*) that were present in northern Africa and humped types (*Bos indicus*) first introduced into Africa from southwest Asia about 3,000 to 5,000 yr ago. The Tuli breed was developed relatively recently in a research program initiated in the 1940's using foundation cattle considered to be the most productive type selected from indigenous Tswana cattle in Zimbabwe. Australian scientists at CSIRO, Tropical Agricultural Research Station, Rockhampton, Queensland, and a consortium of private breeders in Australia imported frozen Tuli embryos from Zimbabwe into Australia in 1990. Semen from nine Tuli bulls has been imported into the U.S. from CSIRO and the consortium of private breeders through an export facility approved by the USDA Animal Plant Health Inspection Service maintained by the Victoria Artificial Breeders Cooperative (VAB) in Australia.

Boran. The Boran was also imported along with the Tuli into Australia from East Africa (Zambia). Borans are a pure zebu breed (*Bos indicus*, humped) that evolved in southern Ethiopia and are believed to have been developed for milk and meat production under stressful tropical conditions. They are believed to have originated from *Bos indicus* cattle imported into Africa about 1,300 to 1,500 yr ago. Semen from eight Boran bulls has been imported into the U.S. from VAB for the experiment.

Belgian Blue. Belgian Blue cattle originate in Belgium. Muscle hyperplasia (double muscling) has been favored for at least 40 yr by Belgian Blue breeders in Belgium. Semen from 26 bulls is being used in the experiment.

Brahman. Semen from a current broad sample of at least 30 Brahman (Grey and Red) bulls will be used to produce F_1 progeny. Semen will also be used from sires sampled in

[1]Cundiff is the research leader, Genetics and Breeding Research Unit, MARC; Gregory is a research geneticist, Genetics and Breeding Research Unit, MARC; Koch is a professor emeritus of animal science, University of Nebraska-Lincoln.

Cycle III of the GPE program (bulls produced in the early 1970's) to facilitate pooling of data and to estimate genetic trends in the breed.

Piedmontese. Piedmontese originate in the Piedmont region of northern Italy. Seventeen Piedmontese sires included in Cycle IV of the program are being repeated to produce one calf crop (1992) in Cycle V.

Calves will be produced in the spring of 1992, 1993 and 1994. At least 100 male and 100 female progeny will be managed following the same experimental procedures as have been used throughout the GPE program. In addition, matings have been made to provide sufficient progeny for intensive nutrition, carcass and meats, reproductive, and biological engineering research studies at MARC.

To evaluate the tropically adapted breeds in subtropical regions of the U.S., cooperative experiments are being conducted at research stations in Oklahoma, Georgia, Florida and three locations in Texas. The mating plans for each location are shown in Table 2.

Results

Preliminary data for calving traits in the first of three calf crops are shown in Table 3. Significant differences were observed among sire breeds for gestation length and birth wt. Differences in calving ease are not significant at this point in the experiment.

As in earlier cycles of the program, progeny of Brahman sires had significantly longer gestation length and heavier birth wt than progeny of Hereford and Angus sires. Progeny of Boran sires, like those sired by Brahman and by other *Bos indicus* breeds reported previously (i.e., Nellore and Sahiwal) had relatively long gestation lengths and heavy birth wt. Progeny of Tuli sires had relatively long gestation length, but significantly lighter birth wt than progeny of any other sire breed. Progeny of Belgian Blue sires had relatively short gestation length, similar to progeny of Hereford and Angus sires. Birth wt of progeny by Piedmontese and Belgian Blue sires ranked between those sired by Hereford and Angus sires. Progeny of Hereford sires tended to have longer gestation length and heavier wt at birth than those by Angus sires.

The 200-day weaning wt of progeny by Brahman sires were significantly heavier than those by Boran and Tuli sires. The relatively heavy 200-day weaning wt of Hereford and Angus sired progeny reflect continuing strong genetic trends for growth rate in these breeds. Belgian Blue sired progeny tended to be heavier than Piedmontese sired progeny in 200-day weaning wt.

Table 1—Sire breeds used in Germplasm Evaluation program at MARC

Cycle I (1970-72)	Cycle II (1973-74	Cycle III (1975-76)	Cycle IV (1986-90	Cycle V (1992-94)
F_1 crosses from Hereford or Angus dams (Phase 2)[a]				
Hereford	Hereford	Hereford	Hereford	Hereford
Angus	Angus	Angus	Angus	Angus
Jersey	Red Poll	Brahman	Longhorn	Tuli
S. Devon	Brown Swiss	Sahiwal	Salers	Boran
Limousin	Gelbvieh	Pinzgauer	Galloway	Belgian Blue
Simmental	Maine Anjou	Tarentaise	Nellore	Brahman
Charolais	Chianina		Shorthorn	Piedmontese
			Peidmontese	
			Charolais	
			Gelbvieh	
			Pinzgauer	
3-way crosses out of F_1 dams (Phase 3)				
Hereford	Hereford			
Angus	Angus			
Brahman	Brangus			
Devon	Santa Gertrudis			
Holstein				

[a] In Cycle V, composite MARC III (1/4 Angus, 1/4 Hereford, 1/4 Pinzgauer and 1/4 Red Poll) cows are also included.

Table 2—Mating plans for evaluation of tropically adapted breeds by location.

Location[a]	Breed of dam[b]	Sire breeds				
		Tuli	Boran	Brahman	Hereford	Angus
MARC, NE	H, A, III	X	X	X	X	X
STARS, FL	A	X		X		
El Reno, OK	HA, A	X	X	X		
Uvalde, TX	A	X		X		
Overton, TX	Bm, A	X		X		X
McGregor, TX	H, A	X	X	X		
Tifton, GA	Crosses	X		X	X	X

[a] Location: MARC denotes the Roman L. Hruska U.S. Meat Animal Research Center, ARS, USDA, in cooperation with the University of Nebraska, Clay Center, Nebraska; STARS denotes the Subtropical Agricultural Research Station, ARS, USDA in Cooperation with the University of Florida, Brooksville, Florida; FRLR denotes the Forage and Livestock Research Laboratory, ARS, USDA in Cooperation with Oklahoma State University, El Reno, Oklahoma; Uvalde, Overton and McGregor denote Texas A & M University, Texas Agricultural Experiment Station, Agricultural Research and Extension Centers at Uvalde, Overton and McGregor, Texas; and Tifton denotes the Coastal Plain Station, Georgia Agricultural Experiment Station, Tifton, Georgia.

[b] A = Angus, H = Hereford, III = Composite MARC III (1/4 Angus, 1/4 Hereford, 1/4 Red Poll, 1/4 Pinzgauer), HA = Angus - Hereford crosses or the reciprocal cross, Bm = Brahman, crosses = rotational crosses of Hereford, Angus and Santa Gertrudis.

Table 3—Breed group means for preweaning traits (preliminary data, 1992 calf crop).

Sire breed of calf	No. calves born	Gestation length days	Calvings unassisted %	Birth wt lb	200-day wean wt lb
Hereford	76	286.3	97.4	99.5	543
Angus	47	284.6	96.1	94.4	522
Tuli	144	291.0	97.9	88.7	496
Boran	125	293.3	95.4	100.7	513
Brahman	136	292.6	92.1	105.6	536
Belgian Blue	135	285.8	94.6	97.7	527
Piedmontese	145	289.9	96.1	96.7	511

Contributions of Ovum Cytoplasm and Uterine Environment and Postnatal Environment to Maternal Effects in Beef Cattle

Keith E. Gregory and Ralph R. Maurer[1]

Introduction

Any contribution or influence on offspring phenotype attributable to its dam, exceeding the inherited sample half of the dam's nuclear genes, is a maternal effect. Maternal effects can be classified into prenatal (e.g., cytoplasmic and uterine components) and postnatal [e.g., lactation, method of rearing (early weaning), plus other postnatal maternal components]. This experiment was designed with two objectives. The first objective was to determine the relative contributions of ovum cytoplasm and uterine influences on prenatal maternal effects by use of embryo transfer (ET). The second objective was to estimate breed differences in prenatal and postnatal maternal effects combined by evaluating differences between reciprocal crosses and the effects of early weaning and to separate the effects of prenatal maternal influences from postnatal maternal influences.

Procedure

Breeds used in this experiment included Red Poll-Angus and Braunvieh-Hereford reciprocal crosses and embryo transfer (ET) into both breeds of each reciprocal cross. In the second part of the experiment, reciprocal cross matings of each pair of breeds was made by natural service. Part of the calves in the second part of the experiment were weaned at 3 to 5 days and reared on a milk replacer and part nursed their dams to an age of 150 to 180 days. Male calves were castrated shortly after birth. All calves produced by embryotransfer (ET) were weaned at 3 to 5 days and reared on a milk replacer and were fed a mixed diet of ground alfalfa hay, rolled oats, cracked corn, soybean meal and molasses (2.8 mCal ME/kg DM and 19.8% CP) to an age of 35 days. Thereafter, they were fed a mixed diet of ground alfalfa hay, corn silage, cracked corn, soybean meal and supplement (2.84 mCal ME/kg DM and 17% CP) to a weight of approximately 250 lb.

Early weaned calves were fed a growing diet of ground alfalfa hay, corn silage, soybean meal and supplement (2.63 mCal ME/kg DM and 14.4% CP) from an average weight of 250 lb to an average weight of 450 lb. Thereafter, females were fed a diet of corn silage, alfalfa haylage and supplement (2.24 mCal ME/kg DM and 12.3% CP) until they were put on improved pasture at an age of 1 yr. All females were retained for breeding.

Castrate males were fed a diet of corn silage, cracked corn, soybean meal and supplement (2.69 mCal ME/kg DM and 12.9% CP) until slaughter at 1052 lb for Red Poll-Angus reciprocal crosses and 1153 lb for Braunvieh-Hereford reciprocal crosses. Individual feed consumption was recorded on each castrate male from approximately 210 days of age until slaughter. Carcass data on castrate males were obtained after a chill period of 24 hr. Standard procedures were used to obtain objective measures and to make subjective evaluations of the traits for which carcass data were collected and analyzed. Soft tissue from the 9-11 rib cut was removed from the bone and ground. Water, dry matter, fat and protein were determined using standard analytical procedures.

Weights were recorded at birth, weaning and every 28 or 56 days during the feeding period of castrate males. For females, weights were recorded at birth, weaning and every 28 or 56 days after weaning until 368 days of age and at 500 days. Thereafter, data on weights, heights and condition scores were recorded on females three times each yr (precalving, prebreeding and when palpated for pregnancy) to an age of 4.5 yr .

Females were retained to produce three calf crops to estimate prenatal maternal effects (ovum cytoplasm and uterine influence) and postnatal maternal effects (reared by breeds of dam differing in maternal ability or weaned at 3 to 5 days) on reproductive and maternal traits and on weight, height and condition score to an age of 4.5 yr.

Results

Neither breed of recipient (uterine influence) nor breed of donor (cytoplasmic influence) had important effects on either growth traits of heifers and steers or on carcass traits of steers. Further, neither breed of recipient nor breed of donor had an effect on percentage conception rate, percentage calf survival, percentage calves produced per cow exposed, or on birth and weaning weights of their progeny. Neither breed of donor nor breed of recipient had an effect on periodic weights, heights and condition scores of females to an age of 4.5 yr. Thus, breed differences in prenatal maternal effects (i.e., ovum cytoplasm and uterine influence) are of little importance for the sample of breeds included in this study.

Large differences in favor of calves that nursed their dams relative to early weaned calves were observed for most growth traits of heifers and steers and in carcass traits of steers. Effects of early weaning were greater in progeny of Braunvieh and Red Poll dams than in progeny of Angus and Hereford dams. Differences between reciprocal crosses favored progeny of Braunvieh and Red Poll dams for growth related traits.

Differences between reciprocal crosses were generally small ($P > .05$) for reproductive, maternal and size related traits of reproducing females, indicating that breed differences in maternal environment provided by breeds that differ greatly in maternal ability do not have long term effects on cow productivity. Even though early weaning resulted in reduced weights and heights, and lower condition scores of cows to an age of 4.5 yr, there was not an effect on cow productivity in regard to percentage conception rate, percentage calf survival, percentage calves produced per cow exposed, and on birth and weaning weights of their progeny.

For greater detail see:

1. Ralph R. Maurer and K. E. Gregory. 1990. Contributions of ovum cytoplasm, uterine environment and postnatal environment to maternal effects in beef cattle. J. Anim. Sci. 68:2319.
2. Keith E. Gregory and R. R. Maurer. 1991. Prenatal and postnatal maternal contributions to reproductive, maternal and size-related traits of beef cattle. J. Anim. Sci. 69:961.

[1]Gregory is a research geneticist, Genetics and Breeding Research Unit, MARC, and Maurer was a research physiologist, Reproduction Research Unit, MARC (deceased December 1989).

Germplasm Utilization in Beef Cattle

Keith E. Gregory, Larry V. Cundiff, Robert M. Koch, and Donald D. Lunstra[1]

Introduction

Heterosis achieved through continuous crossbreeding can be used to increase weight of calf weaned per cow exposed to breeding by 20%. Comprehensive programs of breed characterization have revealed large differences among breeds for most bioeconomic traits. About 55% of the U.S. beef breeding population involving 93% of the farmers and ranchers who produce beef cattle are in production units of 100 or fewer cows. Optimum crossbreeding systems are difficult to adapt in herds that use fewer than four bulls. Further, fluctuation in breed composition between generations in rotational crossbreeding systems can result in considerable variation among both cows and calves in level of performance for major bioeconomic traits unless breeds used in the rotation are similar in performance characteristics. Use of breeds with similar performance characteristics restricts the use that can be made of breed differences in average genetic merit to meet requirements for specific production - marketing situations. The potential of composite breeds as an alternative to continuous crossbreeding for using heterosis and for using genetic differences among breeds to achieve and maintain a more optimum additive genetic (breed) composition needed to be investigated in a comprehensive experiment. The primary objective of this experiment was to estimate the retention of combined individual and maternal heterosis in advanced generations of *inter sè* mated composite populations established with contributions from either four or five breeds. Retention of initial (F_1) heterozygosity after crossing and subsequent random (*inter sè*) mating within crosses is proportional to (n-1)/n when *n* breeds contribute equally to the foundation. When breeds used in the foundation of a composite breed do not contribute equally, percentage of mean F_1 heterozygosity retained is proportional

to $1 - \sum_{i}^{n} P_i^2$, where P_i is the fraction of each of *n* contributing breeds to the foundation of a composite breed. This loss of heterozygosity occurs between the F_1 and F_2 generations, and if inbreeding is avoided, further loss of heterozygosity in *inter sè* mated populations does not occur. A primary question in this experiment was the extent to which retention of heterosis in composite populations is proportional to retention of heterozygosity.

Procedure

Populations. Matings were made to establish three composite populations (MARC I, MARC II, and MARC III) as indicated by Table 1. In this experiment the F1 is defined as the first generation that reflects the final breed composition of a composite population. As indicated by Table 1, F_1, F_2, and F_3 generations were mated *inter sè* to produce, respectively, F_2, F_3, and F_4 generation progeny. Composite populations were originally formed from the same sires and dams that were represented in the nine contributing parental breeds reflected by Table 1. The numbers of sires used and individuals born in each year for each contributing purebreed and for each generation of each composite population are provided by Table 2. Retained heterozygosity relative to F1 generation for different mating types and estimated increase in cow productivity assuming retained heterosis to be proportional to retained heterozygosity is shown in Table 3.

Contributing purebred contemporaries have been maintained for Pinzgauer since 1982 and for all other breeds produced in 1980, 7/8 Pinzgauer (purebred for female animals in breed registry) were produced in 1982, and 15/16 Pinzgauer (purebred for registry of male animals) have been produced since 1984. Pinzgauer females (7/8) producing (15/16) Pinzgauer progeny were included in the analyses.

The Braunvieh population averages between 3/4 and 7/8 Braunvieh and was established by using semen from nine Braunvieh sires originating in Switzerland and the Federal Republic of Germany (Bavaria) on a foundation of purebred (registered and unregistered) Brown Swiss cows. The cows were obtained from dairy herds in Wisconsin and Minnesota as calves in 1967 and 1968. The breed substitution from Brown Swiss to Braunvieh started in 1969. The Simmental, Limousin, Gelbvieh, and Pinzgauer populations were established by mating 20 or more sires of each breed to purebred dams from the same Hereford and Angus populations used in the experiment (except as noted) followed by repeated backcrossing to the four breeds of sire. Grade-up programs to these breeds started at the U.S. Meat Animal Research Center in 1969 for Simmental, in 1970 for Limousin, in 1975 for Gelbvieh, and in 1977 for Pinzgauer. A sample of 3/4 Gelbvieh dams bred to produce 7/8 Gelbvieh progeny was purchased to augment the Gelbvieh population in 1977. The females had been graded up from a female population of Charolais x Angus with the same sample of Gelbvieh sires used in the Gelbvieh grade-up program at the Research Center. The Charolais population was established primarily with the purchase of registered purebred Charolais dams in 1977 and was augmented by Charolais graded-up from an Angus x Hereford base at the Research Center starting in 1967. Charolais sires were sampled from a broad genetic base. The Red Poll population was established from registered dams purchased from several sources in 1966, 1967, and 1968 with sires sampled from a broad genetic base. The Hereford and Angus breeds have been maintained as closed populations (except as noted) since about 1960. A sample of Hereford sires and dams was added in 1966, but this sample did not produce any male progeny that were used to maintain the population. A sample of Angus sires was introduced in 1967 and 1968, but no male progeny produced from these matings were used to maintain the population. Sires used to maintain the purebred populations were descended from males and females used in the foundation of the composite population to which a purebreed contributed. The purebreds have been maintained as registered populations recorded in the appropriate Herd Book of a breed record society. The data included in this study represent the progeny of from 37 to 78 sires of each parental breed and 14 or more sires in each generation of each composite population (Table 2).

Mating Procedure. All yearling heifers were exposed by natural service to yearling bulls (except as noted) for a mating season of 42 days. Since 1987 in Limousin and 1988 in Herefords, bulls 2 or more yr old have been used on yearling

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heifers because of late puberty in both sexes of these breeds. Dams 2 or more yr old were mated by AI for 28 days followed by natural-service exposure for 28 days for a mating season of 56 days. More than 80% of sires have been used in 2 or more yr. From 1978 until 1984, the mating season for yearling heifers was from mid-May until late June and for dams 2 or more yr old was from the first of June until late July. Since 1985, the mating season for yearling heifers has been from late May until near mid-July and for dams 2 or more yr old has been from mid-June until near mid-August. This adjustment of about 2 wk in mating and calving season was made to allow greater synchrony of breeding and calving with nutritive and climatic environment. Nonpregnant animals were retained in all breed groups, unless they were nonpregnant in two successive years, until 1985. Since 1985, all nonpregnant animals have been removed each year from all breed groups. Nonperformance criteria, such as age, color, and extremes in skeletal size, have been used to remove excess cows to maintain population size for each breed group. No females have been removed from the project before exposure to breeding. An attempt has been made to maintain a similar age distribution of dams in each breed group. The F_4 generation of each composite population was removed from the experiment at an age of 1 yr because further loss of heterosis is not expected beyond F_3 generation progeny (Table 1). Genetic expectations for individual and maternal heterosis ($H^I + H^m$) for each generation of each composite population are presented in Table 1.

Dams in each breed group were assigned to sires on a stratified random basis within ages. Half-sib or closer matings were avoided.

The same basic criteria have been used to identify bulls for breeding use in all populations. The intent has been to avoid extremes in regard to weight, condition, and muscular and skeletal anatomy. Avoiding dystocia has been considered in identifying bulls for use in all breed groups. Larger scrotal circumference also has been favored, particularly in breeds that are late to reach puberty (i.e., Hereford and Limousin). Polledness and color patterns of red or red with white markings have been preferred for bulls used in all generations of each composite population. An effort was made to maintain a broad pedigree base in all breed groups. Genetic defects in some breed groups (i.e., "double muscling" in Gelbvieh, MARC I, and MARC II; "parrot mouth" in Gelbvieh and Braunvieh; malocclusion in Hereford, Angus, and Simmental; hydrocephalus in Red Poll and MARC III; and ataxia in Simmental) resulted in some compromise of pedigree breadth by avoiding carriers or close relatives of carriers.

Management of Heifers and Cows. Generally, female populations were fed and managed consistent with their requirements to maintain breed groups in similar condition. The general plan was to group females in three fully integrated management units under the day-to-day supervision of an operations coordinator who had operational responsibility for this project. When a composite population and its contributing parental breeds had similar feed and management requirements they were grouped and managed together: all generations of composite MARC I and Braunvieh, Charolais, and Limousin (Management Group 1); all generations of composite MARC II and Simmental, Gelbvieh, and Pinzgauer (Management Group 2); and all generations of composite MARC III and Hereford, Angus, and Red Poll (Management Group 3). The only deviation from this practice was during the 28-day natural service mating season when all dams were in single-sire mating pastures. The Pinzgauer females were managed with composite MARC II for two reasons: the three management groups had to contain similar numbers of animals and the feed and management requirements of Pinzgauer females are similar to those of Simmental and Gelbvieh. Even though the populations were grouped in the three management groups, efforts were made to apply uniform management protocols among the three units. Types of improved pastures (cool- and warm-season grasses), winter feeding programs, and all basic management practices were the same and were provided consistent with requirements. The sites were contiguous and were without boundaries (i.e., different management groups used the same pastures at different times). All groups received the same feed but the amounts were varied to be consistent with requirements.

Two-year-old dams were fed a mixture of corn silage and alfalfa haylage along with alfalfa and grass hay, starting from 2 to 3 mo before calving and continuing until pastures were adequate to meet their requirements, which was usually in mid- to late April. All older females were fed mixtures of alfalfa and grass hay to meet nutritive requirements, usually from November until mid- to late April. After 1986, economic considerations favored feeding these animals limited quantities of corn silage and alfalfa haylage during winter feeding.

Feeding Young Heifers and Young Bulls. Calves were weaned at an average age of 180 days. Mean birth date was April 7 and calves were weaned the first week of October in most years. After an adjustment feeding period (28 days), heifers were fed diets composed of corn silage, alfalfa haylage, and protein-mineral- vitamin supplement in varying proportions and lengths of time, depending on weather conditions and weight gains of heifers: 1) Period 1, 2.34 Mcal of ME/kg of DM, 11.62% CP; 2) Period 2, 2.24 Mcal of ME/kg of DM, 12.34% CP; and 3) Period 3, 2.18 Mcal of ME/kg of DM, 11.70% CP. Heifers were fed these diets until they were placed on improved cool-season grass pasture from mid- to late April, depending on adequacy to meet nutritive requirements. The three time periods were of approximately equal length. After an adjustment period of 28 days after weaning, intact males were fed a diet composed of corn silage, rolled corn, and protein-mineral-vitamin supplement (2.69 Mcal ME/kg of DM, 12.88% CP) for 140 days.

Data Collection. Calves were weighed at birth, at the middle of the breeding season (end of AI mating period), at weaning, and 28, 84, 140, and 168 days postweaning. Yearling heifers were weighed at the beginning and end of the mating season and when they were palpated for pregnancy. Thereafter, female animals were weighed, measured for height, and scored for condition three times each year (before calving, at the start of the breeding season, and when they were palpated for pregnancy in late October and early November). Observations of estrus were made in yearling heifers starting about March 1 and continuing until the start of the mating season. Yearling heifers were palpated for pregnancy determination per rectum about 2 mo after the end of the mating season and animals 2 or more yr old were palpated about 1 mo after calves were weaned.

Calving difficulty was subjectively evaluated using descriptive scores; i.e., 1 = no difficulty, 2 = little difficulty by hand, 3 = little difficulty with calf jack, 4 = slight difficulty with a calf jack, 5 = moderate difficulty with calf jack, 6 = major difficulty with calf jack, 7 = caesarean birth and 8 = abnormal presentation. Percentage calving difficulty was analyzed (scores 1 and 2 = 0; scores 3, 4, 5, 6 and 7 = 1; and scores of 8 were excluded from analyses). Scores of 8 also were excluded from analysis of calving difficulty score.

Analysis of Data. Data were analyzed by least squares mixed model procedures. The models included the fixed effects of breed group, year, age of dam, and other fixed effects as appropriate. Sire within breed group was included in all models for analysis of all traits as a random effect. Linear functions of means for parental breeds and for each generation of each composite population were computed to estimate retained heterosis. Retained heterosis was estimated from the mean of a composite population minus the mean of the contributing purebreeds weighted by their contribution (1/4 or 1/8) to the composite population. Sire within breed group mean square was used as the error term for linear contrasts to estimate retained heterosis effects.

Results

Heterosis for Growth Traits in Both Sexes. Heterosis effects for birth weight, 200-day weight, 368-day weight, 368-day height, 368-day condition score and 368-day muscling score (males only) were evaluated separately for each sex in F_1, F_2 and combined F_3 and F_4 generations in the three composite populations (Tables 4 and 5). Combined individual and maternal heterosis was significant in the F_1, F_2 and combined F_3 and F_4 generations for each composite population and for the mean of the three composite populations in both sexes for most of the traits evaluated. There was little reduction in heterosis between the F_1 and F_2 generations or between the F_2 generation and the combined F_3 and F_4 generations. In both sexes, mean heterosis retained in combined F_3 and F_4 generations was significantly greater than genetic expectation based on retained heterozygosity for birth weight and for 368-day weight, but did not differ ($P > .05$) from genetic expectation for other traits. These results support the hypothesis that heterosis in cattle for traits related to growth and size is due to dominance effects of genes (Tables 4 and 5).

Heterosis for Puberty Traits in Females and Scrotal Traits of Males. Heterosis effects were evaluated in F_1, F_2, and F_3 generations of females and in the F_1, F_2 and combined F_3 and F_4 generations of males in the three composite populations. Traits included percentage of females reaching puberty at 368, 410, and 452 days, adjusted age, and adjusted weight at puberty and scrotal circumference of males (Table 6). Heterosis was significant for most measures of puberty in each generation of each composite population and for the mean of the three composite populations. Although results are not presented, heterosis for age at puberty was largely independent of heterosis effects on 368-day weight.

Heterosis was significant for scrotal circumference in each generation of each composite population and for the mean of the three composite populations. Heterosis effects on scrotal circumference are mediated both through heterosis effects on growth rate and through factors that are independent of growth rate. There was close agreement in heterosis observed for puberty traits in females and for scrotal circumference in males and genetic expectation based on retained heterozygosity. These results support the hypothesis that puberty traits in females and scrotal circumference in males is due to dominance effects of genes (Table 6).

Heterosis for Birth Weight, Birth Date, Dystocia and Survival as Traits of Dam. Heterosis effects were evaluated as traits of the dam in F_2 progeny of F_1 dams and combined F_3 and F_4 progeny of combined F_2 and F_3 dams in the three composite populations. Traits included birth weight, birth date (Julian), percentage calving difficulty, and percentage survival at birth, 72 hr, and at weaning (Table 7). Effects of heterosis were significant for birth weight for each generation of each composite population and for the mean of the three composite populations. Generally, heterosis effects for percentage calving difficulty were not significant. Effects of heterosis were significant for date of birth (earlier) for each generation of each composite population and for the mean of the three composite populations. Heterosis effects on percentage survival to weaning were positive but generally were not significant. Heterosis retained for birth weight, birth date, and percentage survival in combined F_3 and F_4 generation progeny of combined F_2 and F_3 generation dams did not differ ($P > .05$) from expectation based on retained heterozygosity. These results support the hypothesis that heterosis in cattle for these traits is the result of dominance effects of genes (Table 7).

Heterosis for Reproduction and Maternal Traits. Heterosis effects in F_1 generation dams producing F_2 generation progeny and retained heterosis in combined F_2 and F_3 generation dams producing F_3 and F_4 generation progeny were evaluated. Traits included percentage pregnant, percentage calf crop born, percentage calf crop weaned, 200-day calf weight per female exposed, and 200-day calf weight (Table 8). Also, breed group means and estimates of heterosis of calf crop born based on females palpated pregnant are presented in Tables 9 and 10. Heterosis effects were significant for all traits in F_1 generation females producing F_2 generation progeny for each composite population and for the mean of the three composite populations (Table 8). For 200-day calf weight, heterosis effects were significant for all generations of each composite population and for the mean of the three composite populations. For 200-day calf weight, heterosis retained for the composite MARC II population and for the mean of the three composite populations was greater ($P < .01$) than genetic expectation based on retained heterozygosity.

Heterosis effects for reproductive traits in F_1 generation dams producing F_2 generation progeny were less in composite populations MARC II and MARC III than in composite population MARC I. In composite populations MARC I and MARC II, heterosis retained for reproductive traits in combined F_2 and F_3 generation dams producing F_3 and F4 progeny did not differ from genetic expectation based on retained heterozygosity. In composite population MARC III, loss of heterosis for reproductive traits, other than percentage pregnant, between F_1 generation dams producing F_2 generation progeny and combined F_2 and F_3 generation dams producing F_3 and F_4 generation progeny, was greater than genetic expectation based on retained heterozygosity (Table 8). This greater heterosis loss than genetic expectation for reproductive traits based on retained heterozygosity in composite population MARC III was the result of increased fetal death loss between pregnancy diagnosis and parturition (Tables 9 and 10).

In another major experiment involving Angus, Hereford and Shorthorn, we did not find any evidence of individual heterosis (H^I) for either embryonic or fetal survival but did find that maternal heterosis (H^M) was important for early embryonic survival but not for fetal survival between pregnancy diagnosis and parturition. Results from this experiment do not indicate an effect of heterosis in either the F_1 generation or the combined F_2 and F_3 generations for fetal survival between pregnancy diagnosis and parturition in composite populations MARC I and MARC II (Table 10). Negative recombination effects are suggested for fetal survival between pregnancy diagnosis and parturition in F_1 generation dams 5 or more yr old and in combined F_2 and F_3 generation dams for the three age groups (Table 10).

For composite population MARC III, the F_1 generation as defined in this experiment was produced by reciprocally crossing two single crosses (Table 1). One-half of any losses from the negative effects of recombination of genes are expected in the F_1 generation as defined in this experiment. The negative effects of recombination of genes are generally considered in the context of assumed heterosis. However, negative effects of recombination of genes in descendants of crosses result from loss of favorable epistatic gene combinations that have accumulated and are maintained by either deliberate or natural selection in a parental purebreed. Thus, the presence of heterosis is not required to explain decreased performance in descendants of crosses of parental purebreeds when favorable epistatic gene combinations contribute to the performance of the parental breed(s). These results suggest that combinations of genes with favorable epistatic effects on fetal survival have evolved in either the Red Poll or Pinzgauer breed or possibly both. These combinations are distinctly different from those that have evolved in Hereford, Angus, or other breeds that contributed to composite MARC I or MARC II. The basis for this suggestion is that the Hereford and Angus breeds contribute to all three composite populations, whereas, the Red Poll and Pinzgauer breeds contribute only to composite MARC III.

For composite populations MARC I and MARC II, these results support the hypothesis that heterosis for reproductive and maternal traits in cattle is the result of dominance effects of genes. The same conclusion can be made for maternal traits in composite MARC III (e.g., 200-day calf weight). However, in composite population MARC III these results suggest that favorable epistatic gene combinations contribute to fetal survival between pregnancy diagnosis and parturition in either the Red Poll or Pinzgauer purebreeds, or possibly in both. Evidence suggests that these favorable epistatic gene combinations are recombined in a manner that does not result in a favorable effect on fetal survival in crosses and subsequent *inter sè* matings involving these breeds.

Heterosis on Actual Weight, Adjusted Weight, Hip Height, and Condition Score in Females. Heterosis effects were evaluated in the three composite populations in F_1, F_2 and F_3 generations separately and combined. Because heterosis did not differ ($P > .05$) between generations, only the results from the analysis of combined (F_1, F_2, and F_3) generations from two through seven or more yr old females are presented. Traits included actual weight, weight adjusted to a common condition score, hip height, and condition score (Table 11). The effects of heterosis were generally important ($P < .05$) for all traits in F_1, F_2, and F_3 generations separately and combined in the three composite populations. Although the estimates of heterosis on these traits in one-yr-old females are not presented, generally the magnitude of heterosis observed at one year did not differ from that observed in females from two through seven or more yr old. Thus, heterosis effects on weight did not change after an age of one yr. Adjusting weight to a common condition score resulted in an average reduction of heterosis effects on actual weight by about one-fourth. Thus, about one-fourth of the effects of heterosis on weight results from heterosis effects on condition score. Although estimates of heterosis are not presented separately for each of the three generations of either one-yr-old females or from two through seven or more yr old females of the three composite populations and from the mean of the three composite populations, retained heterosis in the F_3 generation did not differ ($P > .05$) from genetic expectation based on retained heterozygosity. These results support the hypothesis that heterosis for weight, hip height, and condition score of females is the result of dominance effects of genes.

Retained Heterosis for Milk Yield and 200-Day Weight. Retained heterosis in F2 generation females nursing F_3 generation progeny was evaluated in three-, four-, and five or more yr old females. Traits evaluated included 12-hr milk yield, estimated 200-day milk yield, 200-day weight of progeny, and 200-day weight of progeny adjusted to a common estimated milk yield (Table 12). Milk yield was estimated using the weigh/nurse/weigh procedure at intervals of 5 wk when calf age averaged 8, 13, and 18 wk. The effects of heterosis on milk yield were significant for each of the composite populations. Average effects of retained heterosis for the three composite populations on 12-hr milk yield was 1.48 lb (14.5%) and on 200-day weight was 34 lb (6.9%). Adjusting 200-day weight of progeny to a common estimated 200-day milk yield resulted in mean retained heterosis in the three composite populations of 14 lb suggesting that approximately 59% of the retained heterosis effects observed for 200-day weight of progeny was accounted for through retained heterosis effects on milk yield.

Genetic and Phenotypic Variation. Estimates of heritability (h^2) and their standard errors and phenotypic standard deviations (σ_p) were computed separately for purebreds combined and for composite populations combined for all traits evaluated. Estimates of h^2 were computed using the sire within breed-component of variance. Phenotypic standard deviations were computed by extracting the square root of the sum of the between and within sire components of variance. Generally, the differences between purebreds combined and composite populations combined were small and were not consistent for estimates of both h^2 and σ_p. There was no tendency for h^2's or σ_p to be greater for composite populations combined than for contributing purebreeds combined. Thus, greater genetic and phenotypic variation expected for composite populations combined than for purebreeds combined was not observed.

Composite Breed Formation

Concepts and Considerations. The distribution of numbers by herd size in the U.S. beef breeding herd is as follows: 35% represented by herds of 50 cows or fewer; 55% represented by herds of 100 cows or fewer, and 87% represented by herds of 500 cows or fewer. Further, of farms and ranches that have beef cows, 80% have 50 cows or fewer, 93% have 100 cows or fewer and more than 99% have 500 cows or fewer.

With 55% of the U.S. beef breeding herd and 93% of the farms and ranches that have beef cows represented by units of 100 cows or fewer, there are obvious limitations on feasible options for optimum crossbreeding systems. The limitations are most significant if female replacements are produced within the herd and natural service breeding is used. Further, fluctuation between generations in additive genetic (breed) composition in breed-rotation crossbreeding systems restricts the extent to which breed differences in average additive genetic merit for specific characters can be used to match climatic adaptability and performance characteristics to the climatic and nutritive environment and other resources that may be most economical to provide. Thus, the formation of composite breeds based on a multibreed foundation is an attractive alternative, or supplement, to continuous crossbreeding systems to use high levels of heterosis on a continuing basis. Once a new composite breed is formed, it can be managed as a straightbred popu-

lation, and the management problems that are associated with small herd size and with fluctuations between generations in additive genetic (breed) composition in rotational crossing systems are avoided provided there is a source of seedstock (bulls) of the composite breed desired.

Retention of initial heterozygosity after crossing and subsequent random (*inter sè*) mating within the crosses is proportional to (n-1)/n, where *n* is the number of breeds involved in the cross. This loss in heterozygosity occurs between the F_1 and F_2 generations. If inbreeding is avoided, further loss of heterozygosity in an *inter sè* mated composite population does *not* occur. This expression [i.e., (n-1)/n] assumes equal contribution of each breed used in the foundation of a composite breed. Where the breeds used in the foundation of a composite breed do not contribute equally, percentage of mean F_1 heterozygosity retained is proportional to $1 - \sum_{i}^{n} P^2_i$, where P_i is the fraction of each of *n* breeds contributing to the foundation of a composite breed, e.g., heterozygosity retained in a three-breed composite formed from 3/8 breed A, 3/8 breed B and 1/4 breed C can be computed as $1 - [(3/8)^2 + (3/8)^2 + (1/4)^2] = 65.6\%$. Obviously, the maximum number of breeds that can be used to contribute to achieving an optimum additive genetic (breed) composition is preferred because retention of heterozygosity is a function of the number of breeds included in the foundation [i.e., (n-1)/n]. However, use of a greater number of contributing breeds should be balanced against the potential loss in average genetic merit of including the additional breeds. Table 3 provides information on level of heterozygosity relative to the F_1 that is retained after equilibrium is reached for two-, three- and four-breed rotation crossbreeding systems and is presented for two-, three-, four-, five-, six-, seven- and eight-breed composites, with breeds contributing in different proportions in several of the composites. Estimates of increase in weight produced per cow exposed to breeding, based on the assumption that retention of heterosis is approximately proportional to retention of heterozygosity, are presented in Table 3 for each mating type.

Existing breeds of cattle are mildly inbred lines, and because heterosis seems to result primarily from the dominance effects of genes, heterosis can be accounted for as recovery of accumulated inbreeding depression that has occurred in breeds since their formation. Deviation of heterosis from linear association with heterozygosity results from epistatic effects of genes. For loss of favorable epistatic combinations that may either have become fixed or are maintained by either natural or deliberate selection in parental breeds, the deviation from linearity of loss in heterosis with loss in heterozygosity is negative (greater). However, for loss of unfavorable epistatic combinations that may have become fixed through chance, the deviation from linearity of loss in heterosis with loss in heterozygosity obviously is likely to be positive (less). Both genetic situations may exist, but the likelihood is greater for favorable than for unfavorable epistatic combinations in parental breeds, particularly for fitness traits. Also, heterosis may deviate from heterozygosity in a positive direction if a threshold effect (nonlinear) of heterozygosity relative to heterosis should exist.

Other than for characters affected by natural or automatic selection (i.e., fitness), the likelihood is small that fixed favorable epistatic combinations are important because of changing selection goals that have characterized beef cattle breeding.

Because retention of heterosis is, generally, linearly associated with retention of heterozygosity, composite breed formation offers much of the same opportunity as rotational crossbreeding for retaining individual and maternal heterosis, in addition to heterosis in male reproductive performance (Table 3). Further, composite breeds offer the opportunity to use genetic differences among breeds to achieve and maintain the performance level for such traits as climatic adaptability, growth rate and size, carcass composition, milk production, and age at puberty that is optimum for each of a wide range of production environments and to meet different market requirements. Further, composite breeds provide herds of any size with an opportunity to use heterosis and breed differences simultaneously.

A specific composite breed does not permit the use of different genotypes (complementarity) for male and female parents. However, specialized paternal and maternal composite breeds may be developed for use in production systems in which the production resource base and market requirements favor the exploitation of complementarity. Between-breed selection is highly effective for achieving and maintaining an optimum additive genetic composition (performance level) for such specialized breeds by using several breeds to contribute to the foundation population for each specialized composite breed. There is opportunity to develop general purpose composite breeds through careful selection of fully characterized candidate breeds to achieve an additive genetic (breed) composition that is better adapted to the production situation than is feasible through continuous crossbreeding or through intrabreed selection.

The maintenance of effective population size sufficiently large that the initial advantage of increased heterozygosity is not dissipated by re-inbreeding is essential for retention of heterozygosity (heterosis) in composite breeds. *Thus, the resource requirement for development and use of composite breeds as seedstock herds is high, and from an industry standpoint requires a highly viable and creative seedstock segment.* Early re-inbreeding and a small number of inadequately characterized parental breeds contributing to the foundation of composite breeds have likely been major causes for limited success of some previous efforts at composite breed development.

For the seedstock segment that develops composite breeds, it is suggested that the number of females be appropriate for the use of not less than 25 sires per generation. Use of 25 sires per generation would result in a rate of increase in inbreeding of about .5% per generation. With an average generation interval of 5 years, the accumulated inbreeding in a composite breed after 50 years (e.g., 10 generations) would be about 5%. Further, a large number of sires (i.e., 15-20) of each purebreed contributing to a composite breed should be sampled in order to minimize the rate of inbreeding in subsequent generations of *inter sè* mating. Because some of the foundation sires used from each contributing breed are not likely to leave sons, the genetic base will likely be reduced in the first generation. *Inbreeding may be viewed as the "other side of the coin" to heterosis and must be avoided in order to retain high levels of heterozygosity (heterosis) in composite breeds.*

The development of composite breeds may now be viewed as a predictable procedure when contributions are limited to *Bos taurus* breeds. However, because of the dynamic nature of the beef cattle industry, characterization of candidate breeds is needed on a continuing basis in a range of production environments. This information is needed to provide the basis for effective choices of contributing breeds in order to approach the most favorable additive genetic (breed) composition consistent with the role perceived for each composite. *The most appropriate source of this information should be records from perfor-*

mance programs of breed associations that will provide estimates of breed means for major bioeconomic traits on a continuing basis.

Heterosis in crosses of *Bos indicus* breeds with *Bos taurus* breeds is considerably greater (perhaps two fold) than crosses among *Bos taurus* breeds. We do not believe that results from composite populations with contributions limited to *Bos taurus* breeds in regard to linearity of association of heterosis with heterozygosity should be extrapolated to composite breeds that have contributions from both *Bos taurus* and *Bos indicus* breeds. Rather, we believe that a large scale, comprehensive experiment is needed to estimate retention of heterosis in advanced generations of *inter sè* mated composite populations with contributions from both *Bos taurus* and *Bos indicus* breeds.

SUMMARY

Rationale for Development of Composite Breeds

1. Heterosis (hybrid vigor) for major bioeconomic traits including reproduction, calf survival, maternal ability, growth rate and longevity of beef cattle is important. Heterosis can be used to increase weight of calf weaned per cow exposed to breeding by 20%.

2. Large differences exist among breeds of beef cattle for major bioeconomic traits including growth rate and size, composition of gain, milk production, dystocia, (calving difficulty), age at puberty and climatic and nutritive adaptability.

3. About 55% of the cows in U.S. beef breeding herd are in units of 100 or fewer cows. This involves about 93% of the farms and ranches that have beef cows.

4. Crossbreeding systems may be used to achieve high levels of heterosis. However, optimum crossbreeding systems are difficult to adapt in herds that use fewer than four bulls.

5. Fluctuation in breed composition between generations in rotation crossbreeding systems can result in considerable variation among cows and calves in level of performance for major bioeconomic traits unless breeds used in the rotation are similar in performance characteristics.

6. Use of breeds with similar performance characteristics restricts the use that can be made of breed differences in average genetic merit for bioeconomic traits. This includes traits such as: (a) growth rate and size, (b) carcass composition, (c) milk yield, and (d) age at puberty.

7. Composite breeds offer opportunity to: (a) use high levels of heterosis on a continuing basis if population size in seedstock herds is sufficiently large to avoid inbreeding, (b) achieve and maintain optimum breed (additive genetic) composition needed to match performance characteristics of the composite breeds to each of a wide range of production situations and to different market requirements, and (c) achieve and maintain uniform performance levels from one generation to the next.

Conclusions from Experimental Results

1. Generally, high levels of heterosis were observed for growth rate, reproduction, and maternal traits including milk production.

2. Heterosis differed among composite populations for some major bioeconomic traits. Results suggest that specific cross heterosis may be important, i.e., level of heterosis for some traits may vary among specific breed crosses.

3. Generally, retained heterosis in advanced generations was equal to, or greater, than expectation based on retained heterozygosity in the three composite populations. Retained heterosis for reproductive traits did not differ from genetic expectation based on retained heterozygosity in composites MARC I and MARC II. There was no heterosis (individual and maternal combined) for fetal survival in composites MARC I and MARC II.

4. Fetal survival between pregnancy diagnosis and calving was less for composite MARC III than for the average of contributing purebreeds. For composite MARC III results suggest that combinations of genes with favorable epistatic effects on fetal survival have evolved in either the Red Poll or the Pinzgauer breed, or possibly in both, that are distinctly different from those that have evolved in Hereford, Angus or the other breeds that contributed to composites MARC I or MARC II. The basis for this suggestion is that the Hereford and Angus breeds contributed to all three composite populations, whereas, the Red Poll and Pinzgauer breeds contributed only to composite MARC III.

5. Results suggest that although there is, generally, a high relationship between retained heterosis and retained heterozygosity the relationship is not linear for all situations; i.e., for some traits and in some breed combinations, retained heterosis *may be greater* or *may be less* than expectation based on retained heterozygosity.

6. Even though results suggest that specific cross heterosis may be of some importance, it is not feasible to have estimates of F_1 heterosis and of heterosis retained in advanced generations of a large number of specific breed combinations in order to choose breeds as contributors to specific composite populations (breeds). Thus, use of *average* values of F_1 heterosis and of retained heterosis in advanced generations of *inter sè* mated composite populations is suggested.

7. These results, generally, support the hypothesis that heterosis in cattle is primarily due to dominance effects of genes. Thus, heterosis in breed crosses can be accounted for as recovery of accumulated inbreeding depression that has occurred in breeds since their formation.

8. Estimates of heritability and phenotypic standard deviations were similar for parental purebreeds combined and for composite populations combined for most bioeconomic traits. Thus, increased genetic variation that may be expected in composite populations relative to contributing purebreeds was not observed.

9. Composite populations (breeds) offer an alternative breeding system that is *generally* competitive with crossbreeding for using heterosis and is easier to manage regardless of size of herd.

10. Composite populations (breeds) offer a procedure that is more effective than continuous crossbreeding for

using genetic differences among breeds to achieve and maintain *optimum* performance levels for major bioeconomic traits on a continuing basis. This includes traits such as: (a) growth rate and size, (b) composition of gain, (c) milk production, (d) climatic and nutritive adaptability, and (e) age at puberty.

For greater detail see:

1. Keith E. Gregory, L. V. Cundiff, and R. M. Koch. 1991. Breed effects and heterosis in advanced generations of composite populations for preweaning traits of beef cattle. J. Anim. Sci. 69:947.
2. Keith E. Gregory, L. V. Cundiff, and R. M. Koch. 1991. Breed effects and heterosis in advanced generations of composite populations for growth traits in both sexes of beef cattle. J. Anim. Sci. 69:3202.
3. Keith E. Gregory, D. D. Lunstra, L. V. Cundiff, and R. M. Koch. 1991. Breed effects and heterosis in advanced generations of composite populations for puberty and scrotal traits of beef cattle. J. Anim. Sci. 69:2795.
4. Keith E. Gregory, L. V. Cundiff, and R. M. Koch. 1991. Breed effects and heterosis in advanced gen birth weight, birth date, dystocia, and survival as traits of dam in beef cattle. J. Anim. Sci. 69:3574.
5. Keith E. Gregory, L. V. Cundiff, and R. M. Koch. 1992. Breed effects and heterosis in advanced generations of composite populations for reproduction and maternal traits of beef cattle. J. Anim. Sci. 70:656.
6. Keith E. Gregory, L. V. Cundiff, and R. M. Koch. 1992. Breed effects and heterosis in advanced generations of composite populations on actual weight, adjusted weight, hip height, and condition score of beef cows. J. Anim. Sci. 70:1742.
7. Keith E. Gregory, L. V. Cundiff, and R. M. Koch. 1992. Effects of breed and retained heterosis on milk yield and 200-day weight in advanced generations of composite populations of beef cattle. J. Anim. Sci. 70:2366.

Table 1—Matings to establish composites, retention of heterozygosity and expected retention of heterosis

			Composite Populations			
			MARC I	MARC II	MARC III	Mean
Parents of F_1 generation[a]			(C x LH) x (B x LA) OR (C x LA) x (B x LH) Reciprocals	(GH) x (SA) OR (GA) x (SH)	(PA) x (RH) OR (PA) x (HR) Reciprocals	
Breed composition of F_1 and subsequent generations			.25B, .25C, .25L .125H, .125A	.25G, .25S .25H, .25A	.25P. .25R .25H, .25A	
F_1 Heterozygosity[b]			.94[d]	1	1	.98
F_2 Heterozygosity			.78	.75	.75	.76
F_3 Heterozygosity			.78	.75	.75	.76
	Dam	Progeny				
Heterosis[c]	F_1	F_2	$.78\ H^i + .94\ H^m$	$.75\ H^i + 1\ H^m$	$.75\ H^i + 1\ H^m$	$.76\ H^i + .98\ H^m$
Heterosis	F_2	F_3	$.78\ H^i + .78\ H^m$	$.75\ H^i + .75\ H^m$	$.75\ H^i + .75\ H^m$	$.76\ H^i + .76\ H^m$
Heterosis	F_3	F_4	$.78\ H^i + .78\ H^m$	$.75\ H^i + .75\ H^m$	$.75\ H^i + .75\ H^m$	$.76\ H^i + .76\ H^m$

[a] Composite populations were established from same animals used in purebred foundation where C = Charolais, L = Limousin, H = Hereford, B = Braunvieh, A = Angus, G = Gelbvieh, S = Simmental, P = Pinzgauer, and R = Red Poll.

[b] Retention of initial (F_1) heterozygosity following crossing and subsequent random mating within the crosses (*inter sè*) is proportional to $1 - \sum_{i}^{n} P2i$, where P_i is the fraction of each of *n* breeds contributing to the foundation of a composite population. Loss of heterozygosity occurs between the F_1 and F_2 generations. If inbreeding is avoided, further loss of heterozygosity does not occur.

[c] H^i denotes individual heterosis expressed by progeny of a given generation and H^m denotes maternal heterosis expressed by their dams assuming that retention of heterosis is proportional to retention of heterozygosity. F_2 progeny express the maternal heterosis (H^m) of their F_1 dam.

[d] .94 instead of 1 because both sires and dams of F_1 generation were one-fourth Limousin.

Table 2—Number of sires used and individuals born by birth year and breed group

Breed group	Number sires	Number indiv. born	Year of birth												
			1978	1979	1980	1981	1982	1983	1984	1985	1986	1987	1988	1989	1990	1991
Red Poll	51	1,322	47	129	109	114	110	109	109	88	80	84	84	87	87	85
Hereford	68	1,491	142	114	101	118	116	109	113	93	100	104	104	102	102	73
Angus	78	2,076	168	167	227	234	216	225	225	98	85	86	86	84	88	87
Limousin	56	1,478	86	127	117	115	117	121	107	99	106	98	105	96	104	80
Braunvieh	58	1,384	105	107	114	112	115	117	114	95	84	81	85	84	86	85
Pinzgauer	37	816					17	72	115	134	78	75	74	76	86	89
Gelbvieh	51	1,214	19	26	50	93	137	163	116	89	90	89	86	85	84	87
Simmental	67	1,410	145	117	111	110	116	113	111	90	88	80	82	82	84	81
Charolais	57	1,421	90	101	118	104	116	108	117	97	99	96	100	90	94	91
MARC I-F_1	20	583	33	87	141	112	107	103								
MARC I-F_2	24	1,081				38	74	121	147	132	145	121	117	100	86	
MARC I-F_3	45	806							41	65	128	116	122	107	108	119
MARC I-F_4	24	401										37	62	84	105	113
MARC II-F_1	17	730	143	198	183	132	74									
MARC II-F_2	28	1,328			48	100	181	223	199	117	110	105	98	82	65	
MARC II-F_3	42	974						42	99	174	115	116	107	105	103	113
MARC II-F_4	25	533									47	74	77	99	112	124
MARC III-F_1	15	556			115	108	118	113	102							
MARC III-F_2	24	925					42	70	129	174	144	112	100	85	69	
MARC III-F_3	31	694								38	73	119	132	118	97	117
MARC III-F_4	14	307											29	62	93	123

Table 3—Heterozygosity of different mating types and estimated increase in performance as a result of heterosis

Mating type	Heterozygosity % relative to F1[a]	Estimated increase in weight weaned per cow exposed[b] (%)
Purebreeds	0	0
Two-breed rotation	66.7	15.5
Three-breed rotation	85.7	20.0
Four-breed rotation	93.3	21.7
Two-breed composite:		
F3 - 1/2A, 1/2B	50.0	11.6
F3 - 5/8A, 3/8B	46.9	10.9
F3 - 3/4A, 1/4B	37.5	8.7
Three-breed composite:		
F3 - 1/2A, 1/4B, 1/4C	62.5	14.6
F3 - 3/8A, 3/8B, 1/4C	65.6	15.3
Four-breed composite:		
F3 - 1/4A, 1/4B, 1/4C, 1/4D	75.0	17.5
F3 - 3/8A, 3/8B, 1/8C, 1/8D	68.8	16.0
F3 - 1/2A, 1/4B, 1/8C, 1/8D	65.6	15.3
Five-breed composite:		
F3 - 1/4A, 1/4B, 1/4C, 1/8D, 1/8E	78.1	18.2
F3 - 1/2A, 1/8B, 1/8C, 1/8D, 1/8E	68.8	16.0
Six-breed composite:		
F3 - 1/4A, 1/4B, 1/8C, 1/8D, 1/8E, 1/8F	81.3	18.9
Seven-breed composite:		
F3 - 3/16A, 3/16B, 1/8C, 1/8D, 1/8E,1/8F, 1/8G	85.2	19.8
Eight-breed composite:		
F3 - 1/8A, 1/8B, 1/8C, 1/8D, 1/8E, 1/8F, 1/8G, 1/8H	87.5	20.4

[a] Retention of initial (F_1) heterozygosity after crossing and subsequent random (*inter sè*) mating within the crosses is proportional to (n-1)/n when *n* breeds contribute equally to the foundation. When breeds used in the foundationof a composite breed do not contribute equally, percentage of mean F_1 heterozygosity retained is proportional to $1 - \sum_{i}^{n} P2_i$, where Pi is the fraction of each of *n* contributing breeds to the foundation of a composite breed. This loss of heterozygosity occurs between the F_1 and F_2 generations, and if inbreeding is avoided, further loss of heterozygosity in *inter sè* mated populations does not occur.

[b] Based on heterosis effects of 8.5 percent for individual traits and 14.8 percent for maternal traits and assumption that retention of heterosis is proportional to retention of heterozygosity.

Table 4—Effects of heterosis on growth traits - females

	Birth weight (lb)	200-day weight (lb)	368-day weight (lb)	368-day height (in)	368-day condition score[a]
Heterosis					
MARC I					
F_1 minus Purebreds	5.3**	40.1**	64.6**	.8**	.8**
F_2 minus Purebreds	5.7**	40.0**	57.3**	.9**	.5**
$F_{3\&4}$ minus Purebreds	6.2**	40.0**	60.4**	1.1**	.4**
Observed minus Expected[b]	2.0*	8.4*	9.9	.4**	-.2*
MARC II					
F_1 minus Purebreds	2.4**	49.0**	56.9**	.8**	.8**
F_2 minus Purebreds	5.3**	25.4**	44.1**	.4**	.5**
$F_{3\&4}$ minus Purebreds	4.2**	31.5**	49.8**	.6**	.4**
Observed minus Expected[b]	2.4**	-5.1	7.0	-.1	-.2*
MARC III					
F_1 minus Purebreds	3.7**	30.2**	50.3**	.7**	.4**
F_2 minus Purebreds	3.7**	33.3**	52.7**	.4**	.5**
$F_{3\&4}$ minus Purebreds	4.6**	25.8**	46.1**	.5**	.4**
Observed minus Expected[b]	1.8	3.1	8.4	.0	.1
Mean Heterosis					
All Composites					
F_1 minus Purebreds	4.0**	39.7**	57.3**	.8**	.7**
F_2 minus Purebreds	4.8**	32.6**	51.4**	.6**	.5**
$F_{3\&4}$ minus Purebreds	5.1**	32.4**	52.0**	.7**	.4**
Observed minus Expected[b]	2.0**	2.2	8.4*	.1	-.1

[a] 9 = highest, 1 = lowest.

[b] Linear contrasts of observed and expected heterosis to test hypothesis that retained heterosis is proportional to retained heterozygosity.

* P < .05.

** P < .01.

Table 5—Effects of heterosis on growth traits - males

	Birth weight (lb)	200-day weight (lb)	368-day weight (lb)	368-day height (in)	368-day condition score[a]	368-day muscling score[a]
Heterosis						
MARC I						
F_1 minus Purebreds	2.2*	32.8**	58.2**	.7**	.4**	.10
F_2 minus Purebreds	4.2**	34.8**	51.8**	.7**	.2**	.02
$F_{3\&4}$ minus Purebreds	4.4**	31.5**	34.4**	.6**	.1 -	.08
Observed minus Expected[b]	2.6*	6.0*	-11.0	.1	-.2*	-.16
MARC II						
F_1 minus Purebreds	3.3**	65.3**	75.0**	1.3**	.4**	.00
F_2 minus Purebreds	6.2**	29.1**	54.7**	.5**	.5**	.04
$F_{3\&4}$ minus Purebreds	5.5**	37.7**	71.7**	.8**	.4**	-.01
Observed minus Expected[b]	3.1**	-11.2**	15.4*	-.1	.1*	-.02
MARC III						
F_1 minus Purebreds	4.0**	37.0**	57.6**	.9**	.4**	.27**
F_2 minus Purebreds	4.6**	38.4**	69.2**	.7**	.4**	.08
$F_{3\&4}$ minus Purebreds	5.1**	32.2**	73.2**	.7**	.2	.14
Observed minus Expected[b]	2.2	4.2	30.0**	.0	-.2	-.06
Mean Heterosis						
All Composites						
F_1 minus Purebreds	3.1**	45.0**	63.5**	.9**	.4**	.12
F_2 minus Purebreds	5.1**	34.2**	58.6**	.6**	.4**	.04
$F_{3\&4}$ minus Purebreds	5.1**	33.7**	59.8**	.7**	.2**	.02
Observed minus Expected[b]	2.6**	-.4	11.5*	.0	-.1	-.07

[a] 9 = highest, 1 = lowest.

[b] Linear contrasts of observed and expected heterosis to test hypothesis that retained heterosis is proportional to retained heterozygosity.

* P < .05.

** P < .01.

Table 6—Effects of heterosis on puberty traits of females and scrotal circumference of males

	Puberty					
	368 days (%)	410 days (%)	452 days (%)	Adjusted age[a] (days)	Adjusted weight[a] (lb)	Scrotal circumference (cm)
Heterosis						
MARC I						
F_1 minus Purebreds	24.2**	23.6**	10.8**	-22**	22**	.9**
F_2 minus Purebreds	22.5**	23.9**	10.2**	-22**	20**	1.1**
$F_{3\&4}$ minus Purebreds[b]	19.5**	21.3**	6.1**	-21**	18**	1.4**
Observed minus Expected[c]	.6	2.7	-2.3	4	0	.7*
MARC II						
F_1 minus Purebreds	29.4**	26.0**	4.3*	-20**	22**	1.6**
F_2 minus Purebreds	22.2**	20.0**	4.1*	-19**	15**	1.0**
$F_{3\&4}$ minus Purebreds[b]	19.9**	17.7**	2.0	-20**	15**	1.3**
Observed minus Expected[c]	-2.1	-1.8	-1.2	5	0	.1
MARC III						
F_1 minus Purebreds	24.3**	21.7**	7.6**	-20**	15**	1.5**
F_2 minus Purebreds	15.7**	14.5**	2.6	-13**	29**	.7**
$F_{3\&4}$ minus Purebreds[b]	10.0**	9.5**	1.9	-11**	29**	.7**
Observed minus Expected[c]	-8.3	-6.8	-3.8	-4	18	-.4
Mean Heterosis						
All Composites						
F_1 minus Purebreds	26.0**	23.8**	7.5**	-21**	20**	1.3**
F_2 minus Purebreds	20.2**	19.5**	5.6**	-18**	22**	.9**
$F_{3\&4}$ minus Purebreds[b]	16.5**	16.1**	3.3*	-17**	20**	1.1**
Observed minus Expected[c]	-3.3	-2.0	-2.4	1	4	.1

[a] Adjusted to 100% puberty basis.

[b] F_4 generation for scrotal circumference only.

[c] Linear contrasts of observed and expected heterosis to test hypothesis that retained heterosis is proportional to retained heterozygosity.

* P < .05.

** P < .01.

Table 7—Effects of heterosis on birth and survival traits of dam - all ages

	Birth weight (lb)	Birth date (Julian)	Calving difficulty (%)	Survival		
				Birth (%)	72 hrs (%)	Weaning (%)
Heterosis						
MARC I						
F_1 minus Purebreds[a]	6.0**	-2.3**	4	-.9	.0	1.2
F_2 & F_3 minus Purebreds[a]	6.0**	-2.4**	1.6	.3	.5	2.5
Observed minus Expected[b]	.4	.3	1.2	.5	—	1.4
MARC II						
F_1 minus Purebreds[a]	5.7**	-2.7**	1.4.6	.6	1.8	
F_2 & F_3 minus Purebreds[a]	5.7**	-1.8**	3.3*	.7	.9	2.6*
Observed minus Expected[b]	.9	-.5	2.1	2	.4	1.0
MARC III						
F_1 minus Purebreds[a]	4.2**	-1.8**	3.2*	1.2	2.3*	3.3**
F_2 & F_3 minus Purebreds[a]	4.4**	-2.7**	.4.3	1.0		.1
Observed minus Expected[b]	.9	1.2	-2.4	-.7	-1.0	-2.7
Mean Heterosis						
All Composites						
F_1 minus Purebreds[a]	5.3**	-2.3**	-.5	.3	1.0	2.1**
F_2 & F_3 minus Purebreds[a]	5.3**	-2.3**	.5	.4	.8	1.7
Observed minus Expected[b]	.7	.3	1.9	.1	-.1	.1

[a] F_1 generation females producing F_2 generation progeny and combined F_2 & F_3 generation females producing F_3 & F_4 generation progeny.

[b] Linear contrasts of observed and expected heterosis to test hypothesis that retained heterosis is proportional to retained heterozygosity.

* P < .05.

** P < .01.

Table 8—Effects of heterosis on reproduction and maternal traits - all ages

	Pregnant (%)	Calf crop born (%)	Calf crop weaned (%)	200 day calf wt/ female exposed (lb)	200 day calf wt (lb)
Heterosis					
MARC I					
F_1 minus Purebreds[a]	7.5**	7.9**	7.8**	65**	36**
F_2 & F_3 minus Purebreds[a]	7.3**	6.4**	6.6**	60**	37**
Observed minus Expected[b]	.8	-.5	-.2	4	5
MARC II					
F_1 minus Purebreds[a]	3.6**	4.0**	5.0*	45**	28**
F_2 & F_3 minus Purebreds[a]	1.0	1.2	2.2	40**	40**
Observed minus Expected[b]	-1.9	-2.0	-1.8	4	16**
MARC III					
F_1 minus Purebreds[a]	5.5**	4.2**	6.2**	56**	36**
F_2 & F_3 minus Purebreds[a]	1.9	-2.6	-2.5	9	31**
Observed minus Expected[b]	-2.6	-6.0*	-7.5**	-36**	1
Mean Heterosis					
All Composites					
F_1 minus Purebreds[a]	5.5**	5.4**	6.3**	55**	33**
F_2 & F_3 minus Purebreds[a]	3.4**	1.7	2.1	37**	36**
Observed minus Expected[b]	-1.2	-2.8*	-3.1*	-9	7**

[a] F_1 generation females producing F_2 generation progeny and combined F_2 & F_3 generation females producing F_3 & F_4 generation progeny.

[b] Linear contrasts of observed and expected heterosis to test hypothesis that retained heterosis is proportional to retained heterozygosity.

+ P < .10.

* P < .05.

** P < .01.

Table 9—Breed group means for percentage calf crop born based on females palpated pregnant

		Number	Two years old	Number	Five or more years old	Number	All ages
Overall mean		4,744	96.2	5,153	96.4	16,820	96.3
Red Poll		305	95.1	338	96.1	1,127	93.9
Hereford		260	97.2	461	98.1	1,200	96.7
Angus		476	96.8	601	96.3	1,736	95.8
Limousin		254	97.1	422	99.1	1,207	98.1
Braunvieh		316	96.8	338	97.6	1,130	96.9
Pinzgauer		285	97.2	94	96.6	759	97.0
Gelbvieh		344	97.2	185	96.7	941	97.6
Simmental		344	98.6	297	96.6	1,110	97.3
Charolais		306	93.0	330	98.8	1,173	97.1
Parental breed mean			96.6		97.3		96.7
D.05[a]			4.9		4.2		5.0
MARC I	F_1[b]	175	96.0	523	99.1	1,070	97.8
	F_2&F_3[b]	394	96.8	145	97.2	946	96.3
MARC II	F_1[b]	242	96.5	640	97.3	1,369	97.5
	F_2&F_3[b]	461	96.0	273	96.8	1,282	97.0
MARC III	F_1[b]	202	96.6	440	93.6	989	94.6
	F_2&F_3[b]	380	91.5	66	85.6	781	90.7
D.05[c]			5.4		4.7		5.5

[a] D.05 is the approximate difference between means of parental breeds required for significance.

[b] F_1 generation females producing F_2 generation progeny and combined F_2 & F_3 generation females producing F_3 & F_4 generation progeny.

[c] D.05 is the approximate difference between means of all breed groups required for significance.

Table 10—Effects of heterosis on percentage calf crop born based on females palpated pregnant

	Two years old	Five or more years old	All ages
Heterosis			
MARC I			
F_1 minus Purebreds[a]	0	1.0	.7
F_2 & F_3 minus Purebreds[a]	.8	-1.0	-.8
Observed minus Expected[b]	.8	-1.9	-1.4
MARC II			
F_1 minus Purebreds[a]	-1.0	.3	.7
F_2 & F_3 minus Purebreds[a]	-1.4	-.2	.1
Observed minus Expected[b]	-.6	-.4	-.5
MARC III			
F_1 minus Purebreds[a]	0	-3.2**	-1.3
F_2 & F_3 minus Purebreds[a]	-5.1**	-11.2**	-5.1**
Observed minus Expected[b]	-5.1**	-8.6**	-4.0**
Mean Heterosis			
All Composites			
F_1 minus Purebreds[a]	-.3	-.6	0
F_2 & F_3 minus Purebreds[a]	-1.9*	-4.1**	-2.0**
Observed minus Expected[b]	-1.6+	-3.6**	-2.0**

[a] F_1 generation females producing F_2 generation progeny and F_2 & F_3 generation females producing F_3 & F_4 generation progeny.

[b] Linear contrasts of observed and expected heterosis to test hypothesis that retained heterosis is proportional to retained heterozygosity.

+ $P < .10$.

* $P < .05$.

** $P < .01$.

Table 11—Effects of heterosis on weight, height and condition score – two through seven or more years old with composite generations combined

	Actual weight (lb)	Adjusted weight[a] (lb)	Height (in)	Condition score[b]
Linear contrasts				
Heterosis				
MARC I				
F_1, F_2 & F_3 minus purebreds	46**	34**	.4**	.4**
MARC II				
F_1, F_2 & F_3 minus purebreds	20**	12**	.2*	.2**
MARC III				
F_1, F_2 & F_3 minus purebreds	61**	45**	.4**	.3**
Mean heterosis				
All composites	42**	30**	.3**	.3**

[a] Adjusted to a common condition score.

[b] 9 = highest, 1 = lowest

* $P < .05$.

** $P < .01$.

Table 12—Effects of retained heterosis on milk yield and 200-day weight of progeny

	12-hour milk yield (lb)	Estimated 200-day milk yield (lb)	200-day weight of progeny (lb)	Adjusted 200-day weight of progeny[a] (lb)
Linear contrasts				
Heterosis				
MARC I[b] minus purebreds	1.78**	719**	36**	14*
Percent heterosis	17.1	16.7	7.3	2.7
MARC II[b] minus purebreds	1.25**	504**	41**	22**
Percent heterosis	12.1	11.9	8.2	4.7
MARC III[b] minus purebreds	1.40**	499**	26**	7
Percent heterosis	14.2	12.1	5.1	1.5
Mean heterosis				
All composites				
Composites[b] minus purebreds	1.48**	574**	34**	14*
Percent heterosis	14.5	13.6	6.9	3.0

[a] Adjusted to a common estimated milk yield.

[b] F_2 generation females nursing F_3 generation progeny.

* $P < .05$.

** $P < .01$.

Use of Crossbreeding and Breed Differences to Meet Specific Targets for Production and Carcass Traits of Beef Cattle

Keith E. Gregory, Larry V. Cundiff, and Robert M. Koch[1]

Introduction

The specific requirements for effective use of breed differences to meet specific production and market requirements are: (1) accurate assessment of production resources in regard to availability and costs, (2) accurate assessment of market requirements; i.e., value differences in carcass composition associated with yield grade and quality grade, and (3) accurate current characterization of breeds in regard to such traits as: (a) growth rate and size, (b) carcass composition, (c) milk production, and (d) age at puberty. This information is needed to identify contributing breeds to use in alternative mating systems to achieve specific targets for production and carcass traits. The objective of the beef cattle industry is to synchronize production and carcass characteristics of breed resources with the production resources that are most economical to provide in order to maximize *economic efficiency*.

Information on breed differences is presented in another paper in this report, e.g., "Differences Among Parental Breeds in Germplasm Utilization Project."

The large differences that exist among breeds for most bioeconomic traits are the result of different selection goals in different breeds. Results from the Germplasm Evaluation Program at the U.S. Meat Animal Research Center provide evidence that genetic variation between breeds is of a similar magnitude to genetic variation within breeds for many bioeconomic traits. The heritability of breed differences approaches 100%, whereas, the heritability of differences within breeds for major bioeconomic traits varies from less than 10% to about 50%, depending on the trait. Heritability of breed differences approach 100% because estimates of breed differences are based on the means of a large number of individuals from a representative sample. This results in averaging genetic differences between individuals within breeds. Estimates of heritability of differences within breeds are generally based on single observations of individuals for a specific trait. Thus, selection among breeds is considerably more effective than selection within breeds.

Breed differences in bioeconomic traits are an important genetic resource and can be used to achieve and maintain performance levels that are optimum for different production and marketing situations. *In addition to using breed differences to optimize production and carcass traits or to meet specific targets, the mating system should be organized to achieve and maintain high levels of heterosis or hybrid vigor.*

Alternative Mating Systems

Genetic variation in alternative mating systems is shown in Figure 1 expressed in genetic standard deviation units. Panel 1 (Figure 1) shows that genetic variation between breeds is approximately equal to genetic variation within breeds for some bioeconomic traits. For example, mean percentage retail product of Hereford or Angus is approximately six genetic standard deviation units less than mean percentage retail product for Charolais, Limousin and Chianina.

Panel 2 (Figure 1) shows the difference between generations at equilibrium in rotation crosses of two pure breeds that have a mean difference in a bioeconomic trait of six genetic standard deviation units. The optimum varies in different production and market situations for such traits as: (1) growth and size, (2) milk production, (3) carcass composition, and (4) age at puberty and is reflected by zero in Figure 1. If the mean of the two breeds is optimum, then one-half of the cattle would be more than one genetic standard deviation from the optimum in a rotational crossbreeding system of two pure breeds whose means differ by six genetic standard deviation units. Retained heterosis at equilibrium for a continuous two-breed rotation crossbreeding system is 67% of the F_1 level.

Another alternative is rotational crossbreeding of F_1 males. This alternative has some inherent long-term advantages. Inter-generation variation (Figure 1, panel 2) can be minimized in commercial production if breeds chosen to produce F_1's are selected to optimize performance levels in the F_1 cross. Panel 3 (Figure 1) reflects the genetic variation expected with rotational crossing of AB and CD F_1's where A and C represent a common biological type and B and D another common biological type. Then, performance is optimized in each F_1 (AB = CD) and in their rotational cross (AB-CD). Panel 3 (Figure 1) also depicts the genetic variation expected in rotational crossing of F_1 males having one breed in common (e.g., AB-AD, where B and D are the same biological type).

Panel 3 (Figure 1) shows that rotational crossbreeding using two different F_1's (e.g., AB-CD or AB-AD) or a composite breed based on equal contribution by each of four breeds (e.g., ABCD) can result in populations that have about two-thirds of the animals within one genetic standard deviation of the optimum. The retained heterosis at equilibrium in a continuous rotation of sires using two different F_1's (e.g., AB-CD) is 83.5% of the F_1 level. The retained heterosis at equilibrium in continuous rotation of sires from two F_1's having one breed in common (e.g., AB-AD) is 67% of the F_1 level. The retained heterosis in a four breed composite with breeds contributing equally (e.g., ABCD) is 75% of the F_1 level provided the population is sufficiently large to avoid inbreeding.

Genetic variation in a composite breed with equal contributions by four breeds is approximately equal to continuous rotation of sires using two different F_1's that are approximately equal (e.g., AB=CD or AB=AD), (Panel 3).

Thus, a rotational crossbreeding system using F_1 males produced from different breeds (e.g., either AB-CD or AB-AD) is preferred to a rotational crossbreeding system using two pure breeds for using breed differences to achieve a more optimum additive genetic (breed) composition. It is either superior or equal to a continuous two-breed (67%) rotational crossbreeding system for using heterosis. Similarly, a continuous rotational crossbreeding system using F_1 males of different breeds can be competitive with a composite breed based on equal contribution by four breeds for using both heterosis and breed differences to achieve an optimum additive genetic (breed) composition.

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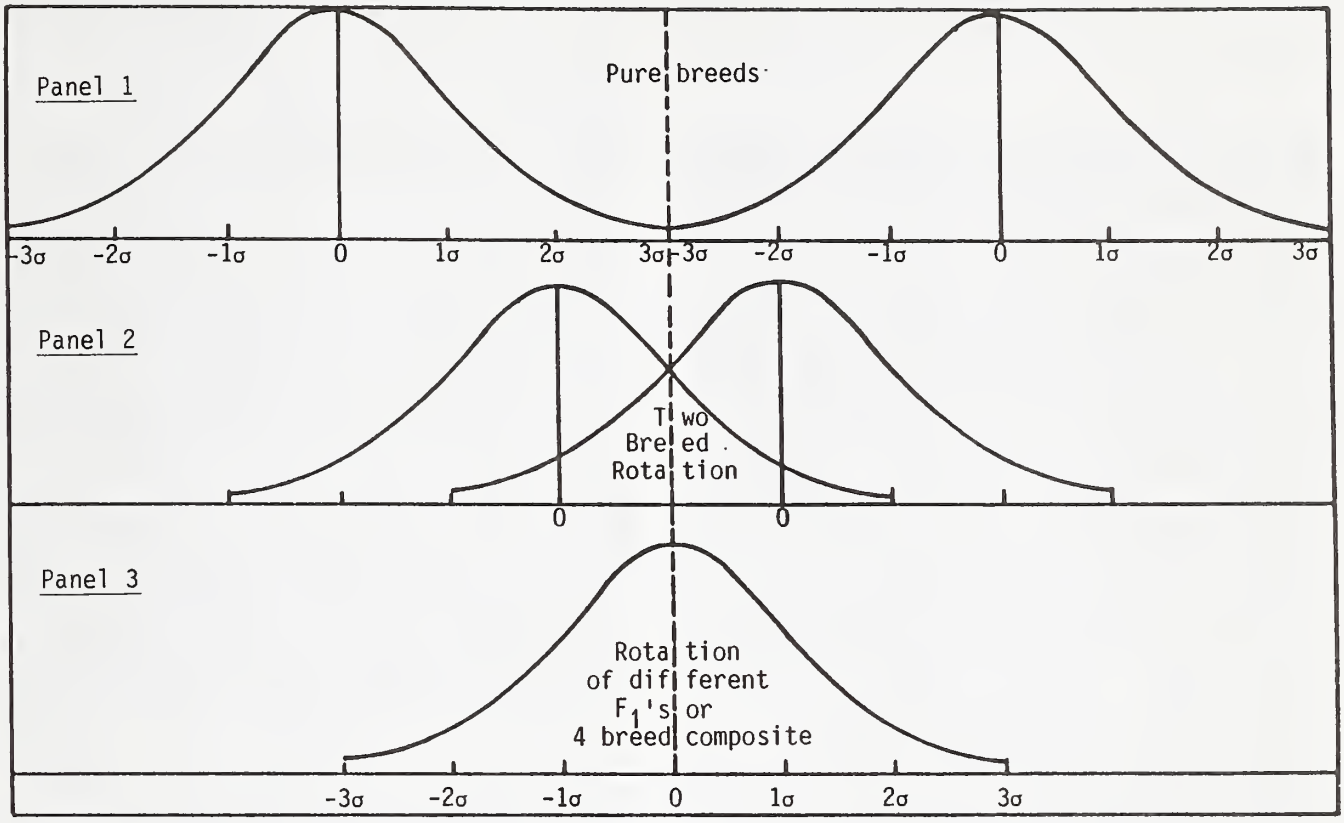

OPTIMUM

ASSUMES THAT THE TWO F_1's USED ARE OF SIMILAR GENETIC MERIT.

Differences Among Parental Breeds in Germplasm Utilization Project

Keith E. Gregory, Larry V. Cundiff, Robert M. Koch, and Donald D. Lunstra[1]

Introduction

Large differences exist among breeds for most bioeconomic traits. These differences are the result of different selection goals in different breeds. Thus, over time, large genetic differences have accumulated among breeds. Results from the Germplasm Evaluation Program at the U.S. Meat Animal Research Center provide evidence that genetic variation between breeds is of similar magnitude to genetic variation within breeds for many bioeconomic traits. However, the heritability of breed differences approaches 100%, whereas, the heritability of differences within breeds for major bioeconomic traits varies from less than 10% to about 50% depending on the trait. Heritability of breed differences approaches 100% because estimates of breed differences are based on the means of a large number of individuals from a representative sample. This tends to average within breed variation. Estimates of heritability of differences within breeds are generally based on a single observation of individuals for a specific trait. Thus, selection among breeds is much more effective than selection within breeds. Breed differences in bioeconomic traits are an important genetic resource and can be used to achieve and maintain performance levels that are optimum for different production-marketing situations for such traits as: (1) growth rate and size, (2) milk production, (3) carcass composition, (4) age at puberty, and (5) climatic and nutritive adaptability. Large breed differences exist for these traits and breed differences may be used to achieve and maintain optimum additive genetic (breed) composition through the formation of composite breeds or through the use of specific crossbreeding systems.

Procedure

The results presented on differences among breed group means are from the Germplasm Utilization Project. The procedures of this experiment including analysis of data are presented in the paper, "Germplasm Utilization in Beef Cattle" in this report.

The number of animals in each breed group born in each year is presented in Table 1. Adjustment factors for age of dam to a mature equivalent basis (5 or more yr) for each breed group are presented for each sex for growth traits in Table 2.

Results

Differences Among Parental Breeds for Growth Traits (Males and Females)

Differences among parental breeds reported here include the sum of the additive direct and additive maternal genetic effects ($G^i + G^m$). The effects of breed group were important ($P < .01$) for all growth traits evaluated in females (Table 3) and in males (Table 4). Age of dam adjustment factors to a mature basis (5 or more yr) for birth weight, preweaning average daily gain, and postweaning average daily gain for each sex and each breed group are presented in Table 2. For weights presented in this study, values were adjusted to a mean age of dam of 3.5 yr; (i.e., 2- 3- 4-, 5 or more yr).

The approximate differences between means, of parental breeds and of all breed groups, required for significance are presented in Tables 3 and 4 for females and males, respectively. Means for birth weight of females ranged from 71.9 lb in Angus to 97.7 lb in Pinzgauer (Table 3) and in males ranged from 77.0 lb in Angus to 108.0 lb in Pinzgauer (Table 4). Means for 200-day weight of females ranged from 392 lb in Hereford to 536 lb in Simmental (Table 3) and in males ranged from 419 lb in Hereford to 571 lb in Gelbvieh (Table 4). Means for 368-day weight of females ranged from 631 lb in Hereford to 787 lb in Simmental and Charolais (Table 3) and in males ranged from 842 lb in Hereford to 1,052 lb in Simmental (Table 4). Means for hip height of females at 368 days ranged from 44.1 in. in Hereford to 48.8 in. in Braunvieh, Gelbvieh, Simmental and Charolais (Table 3) and in males ranged from 45.7 in. in Hereford to 50.8 in. in Simmental (Table 4). Means for condition score in females at 368 days ranged from 3.7 in Limousin to 5.9 in Angus (Table 4) and in males at 368 days ranged from 3.3 in Limousin to 5.6 in Hereford and Angus (Table 4). Means for muscle score in males at 368 days ranged from 4.0 in Red Poll to 6.9 in Limousin. Muscle score was not recorded in females at 368 days.

The range of parental breed differences of each sex of more than 35% in birth weight, more than 35% in 200-day weight and more than 24% in 368 day weight reflects great opportunity to select among breeds for differences in growth traits in combined additive direct (G^i) and additive maternal (G^m) genetic effects.

Differences Among Parental Breeds for Puberty Traits of Females and Scrotal Circumference of Males

Differences among parental breeds reported here include the sum of additive direct and additive maternal genetic effects ($G^i + G^m$).

Puberty of Females. Means are presented by breed group in Table 5 along with approximate difference between means, of parental breeds and of all breedgroups, required for significance. Percent in parental breeds reaching pubertyat 368 days (end of feeding period), at 410 days (start of breeding season) and at 452 days (end of breeding season) was, respectively, 65.3 (ranged from 31.7 in Hereford to 89.7 in Braunvieh), 72.9 (ranged from 39.9 in Hereford to 94.2 in Braunvieh), and 92.6 (ranged from 79.3 in Limousin to 100 in Braunvieh). Parental breed differences in adjusted age at puberty ranged from 350 days in Braunvieh to 411 days in Hereford with a mean of 376 days. This range of 61 days between breed group means is of major importance when females are exposed for mating as yearlings in a restricted mating season of 42 days. Of equal interest and significance is the difference among breeds in percent that had reached puberty at the start of the breeding season (410 days). This parental breed difference (39.9% vs 94.2%) is more than twice as great as the heterosis effect on percent reaching puberty at the start of the

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breeding season (410 days). These results suggest a high relationship among breeds between age at puberty and breed history of selection for milk production.

Parental breed differences in adjusted age and weight at puberty reported here are likely underestimated because observations on date of first estrus were not started until March 1. We can assume that a higher percentage of breed groups that reach puberty at younger ages had already reached puberty before observations for estrus were started than for breed groups that reach puberty later. Cumulative percent that reached puberty by 368, 410, and 452 days are not as likely to be underestimated. Thus, greater attention should focus on differences among cumulative percentages that reached puberty by 368, 410, and 452 days than on differences in adjusted age at puberty.

Results from a specific analysis indicated that breed group differences in age at puberty are largely independent of breed group differences in 368-day weight.

Scrotal Circumference of Males. Means are presented by breed group in Table 5 along with approximate difference between means, of parental breeds and of all breed groups, required for significance. Mean scrotal circumference adjusted for age of dam (linear, .434 and quadratic, -.084) by regression (3.6 yr) and for date of birth (linear, -.043) by regression (354 days) was 32.4 cm for all parental breeds. Differences between breeds in scrotal circumference at 368 days ranged from 29.0 cm in Limousin to 34.1 cm in Gelbvieh.

Significant differences among breed groups remained in scrotal circumference after adjusting scrotal circumference by regression for differences in 368-day weight. This result suggests that differences among breed groups in scrotal circumference are influenced only in part by breed group differences in 368-day weight.

Correlations. Correlation coefficients among breed group means for puberty traits in females with scrotal circumference of males were .88 ($P < .01$) or higher, (Table 6). Correlations coefficients among breed group means for puberty traits in females with pregnancy percentage as yearlings were .87 ($P < .01$) or higher (Table 7).

General. These results reveal large differences among breeds in percent reaching puberty at 368, 410 and 452 days and in age at puberty of females and in scrotal circumference of males and show a high correlation among breed group means for these traits. Breed differences in these traits provide considerable opportunity to use genetic differences among breeds to optimize additive genetic value to meet a wide range of production situations.

Adjusting female age at puberty for differences in 368-day weight by regression had little effective on breed rank or variation among breeds for age at puberty, suggesting that differences in age at puberty among breeds is largely independent of breed differences in growth rate to 368 days. Adjusting scrotal measurements for differences in 368-day weight by regression resulted in some reduction in variation among breeds in scrotal circumference (range reduced from 5.1 cm to 3.7 cm). However, significant breed differences in scrotal circumference were still present. Although differences in scrotal circumference among breeds are partially attributable to breed differences in 368-day weight, there are important ($P < .01$) differences among breeds in scrotal circumference independent of breed differences in growth ratendent of weight.

Differences Among Parental Breeds for Birth Weight, Birth Date, Dystocia and Survival - as Traits of Dam.

Differences among parental breeds include additive direct genetic effects and additive maternal genetic effects ($G^I + G^m$).

Calves With 2 Year Old Dams. Mean birth weight for parental breed calves was 83.1 lb. Birth weight ranged from 69.4 lb in Angus to 93.3 lb in Braunvieh ($P < .01$), (Table 8). Mean birth date (Julian) for parental breed calves was 79. Julian birth date ranged from 74 in Red Poll and Pinzgauer to 88 in Limousin ($P < .01$), (Table 8). Breed rank for birth date is highly associated with age at puberty and (or) gestation length. Mean calving difficulty percentage (percentage requiring assistance) for parental breeds was 52.6%. Calving difficulty ranged from 31.8% in Angus to 73.8% in Braunvieh ($P < .01$), (Table 8). Mean calf survival at weaning for parental breeds was 80.4%. Survival at weaning ranged from 74.3% in Hereford to 85.4% in Red Poll ($P < .05$), (Table 8). Results from a separate analysis showed the effects of breed group were important ($P < .01$) for calving difficulty percentage and survival percentage at birth, 72 hr and at weaning independent of breed group effects on birth weight. The effects of sex were important, independent of the effects of sex on birth weight, ($P < .01$) for calving difficulty percentage; (e.g., males = 62% and females = 46% adjusted to a common birth weight).

These results reveal that in 2 yr old dams, each lb increase in birth weight resulted in a 1.9% increase in calves requiring assistance and that the response was linear. The negative linear and quadratic regressions ($P < .01$) of survival percentage at birth, 72 hr and weaning on birth weight reflect the importance of birth weight on calf survival percentage and the curvilinearity of the effect. The significant effect of breed group on calving difficulty % and survival % at birth, 72 hr and weaning adjusted to a common birth weight reflect important differences among breed groups for these traits, independent of breed group effects on birth weight. Thus, there appears to be some opportunity to reduce dystocia and to increase calf survival % by consideration of factors other than birth weight such as anatomical characteristics of dam and(or) calf. Similarly, the greater calving difficulty % of males vs females at a common birth weight document important effects of sex on calving difficulty %, independent of sex effects on birth weight.

Calves With Dams 3 or More Years Old. Mean birth weight of parental breed calves with dams > 3 yr old was 92.6 lb. Birth weight ranged from 76.3 lb in Angus to 104.5 lb in Pinzgauer ($P < .01$), (Table 9). Mean birth date (Julian) of parental breed calves with dams 3 or more yr old was 105. Julian birth date ranged from 99 in Angus tc 109 in Limousin and Braunvieh ($P < .01$), (Table 9). Mean calving difficulty of parental breed calves with dams 3 or more yr old was 8.6%. Calving difficulty ranged from .9% in Angus to 15.9% in Pinzgauer ($P < .01$), (Table 9). Mean calf survival at weaning of parental breed calves with dams or more 3 yr old was 92.6%. Survival at weaning ranged from 88.1% in Simmental to 95.6% in Red Poll ($P < .01$), (Table 9).

In a separate analysis, gestation length, birth date, birth weight, calving difficulty percentage, survival percentage at birth and survival percentage at 72 hr were analyzed for calves with 3 or more yr old dams. Data on gestation length were not available on calves with 2 yr old dams. Breed group effects were significant for gestation length, birth date, birth weight and calving difficulty percentage but not for survival percentage at birth and at 72 hr. A regression analysis of these traits on gestation length, gestation length

within sex and gestation length within breed group was conducted. The analyses revealed the linear regressions of all traits on gestation length (days) to be significant; e.g., birth date, 1 day; birth weight, .9 lb, survival at birth, .1% and survival at 72 hr, .2%. The regressions of all traits, except birth weight, on gestation length within sex were significant. The regressions of all traits, except birth date, on gestation length within breed group were significant. Gestation length accounted for 90% of the breed group variation in birth date, 14% of the breed group variation in birth weight and 31% of the breed group variation in calving difficulty percentage.

Calves With Dams of All Ages. Mean birth weight of parental breed calves with dams of all ages was 90.2 lb. Birth weight ranged from 74.7 lb in Angus to 101.9 lb in Pinzgauer ($P < .01$), (Table 10). Mean birth date (Julian) of parental breed calves with dams of all ages was 99. Julian birth date ranged from 93 in Angus to 104 in Limousin ($P < .01$), (Table 10). Mean calving difficulty percentage of parental breed calves with dams of all ages was 19.7. Calving difficulty ranged from 8.8% in Angus to 28.5% in Braunvieh ($P < .01$), (Table 10). Mean survival at weaning of parental breed calves with dams of all ages was 89.7%. Survival at weaning ranged from 86.4% in Simmental to 93.4% in Red Poll ($P < .01$), (Table 10).

General. These results show large differences among breeds in calving difficulty, particularly in calves with 2 yr old dams. While the means are not presented here, calves with difficult births with 2 yr old dams were significantly heavier at birth and had significantly lower survival percentage at 72 hr and at weaning than calves with 2 yr old dams that did not experience difficult births. Large differences were observed among breed groups in calving difficulty percentage and calf survival percentage independent of breed group effects on birth weight. This result suggests some opportunity to reduce dystocia and to increase calf survival percentage by consideration of factors other than birth weight such as anatomical characteristics of dam and(or) calf. Similarly, greater calving difficulty percentage was observed in male calves than in female calves, independent of sex effects on birth weight, indicating that anatomical differences between sexes likely contribute to dystocia. In 2-yr-old females calf survival percentage at weaning was lowest ($P < .05$) in smallest and in largest birth weight classes and did not differ ($P > .05$) among intermediate birth weight classes. These results document that intermediate birth weights are optimum for increased survival.

Differences Among Parental Breeds for Reproduction and Maternal Traits

Differences among parental breeds include additive direct genetic effects and additive maternal genetic effects ($G^I + G^m$).

Because of the importance of breed differences associated with age for some of the traits evaluated, results are presented for three age groupings: two yr old, five or more yr old and in females of all ages.

Two Year Old Females. Means are presented by breed group in Table 11 along with approximate difference between means, of parental breeds and of all breed groups, required for significance.

Large differences ($P < .01$) were observed among parental breeds for pregnant percentage when bred as yearlings in a 42 day mating season. Differences ranged from 54.7% in Limousin to 85.6% in Gelbvieh. Breed group means in pregnant percentage as yearlings were highly associated ($P < .01$) with breed group means in measures of puberty (Table 7). Large differences ($P < .01$) were observed among parental breeds in calf crop born percentage. Differences between pregnant percentage and calf crop born percentage (e.g., 2.6%) reflect both errors in diagnosing pregnancy by rectal palpation and fetal losses between pregnancy diagnosis and parturition. Rank of parental breeds for calf crop born percentage was similar to rank of breeds for pregnant percentage with Limousin lowest (53.0%) and Gelbvieh highest (83.2%). Large differences ($P < .01$) were observed among parental breeds in calf crop weaned percentage. Differences between calf crop weaned percentage and calf crop born percentage reflect calf mortality between birth and weaning (e.g., 13.7% including calves dead at birth). Rank of parental breeds for calf crop weaned percentage was similar to rank of breeds for calf crop born percentage with Limousin lowest (41.8%) and Braunvieh highest (66.4%).

Large differences ($P < .01$) were observed in 200-day calf weight per female exposed to breeding. These values reflect differences among parental breeds in both calf crop weaned percentage and 200-day calf weight; i.e., reproduction rate, calf survival and preweaning growth for additive genetic maternal effects (G^m) and additive direct genetic effects (G^I). Because of the importance of fitness traits in contributing to this measure of output per female, there was considerable similarity in breed rank for 200-day calf weight per female exposed and measures of fitness; i.e., reproduction rate and calf survival. Hereford ranked lowest (178 lb) and Gelbvieh ranked highest (340 lb) in 200-day calf weight per female exposed to breeding.

Large differences ($P < .01$) were observed in 200-day calf weight with Hereford lowest (378 lb) and Gelbvieh highest (506 lb). These values include breed differences in additive maternal genetic effects (G^m) and additive direct genetic effects (G^I) for preweaning growth.

Females Five or More Years Old. Means are presented by breed group in Table 12 along with approximate difference between means, of parental breeds and between means of all breed groups, required for significance. Because of the large differences among breeds in reproductive traits of two yr old females associated with differences in age at puberty (Table 7) it was desirable to evaluate breed differences in reproductive and maternal traits after they were mature; e.g., 5 to 10 yr at parturition.

Parental breed differences were large ($P < .05$) for 200-day calf weight per female exposed with Hereford lowest (348 lb) and Charolais highest (477 lb). However, Charolais, Braunvieh, Gelbvieh, Simmental and Pinzgauer did not differ ($P > .05$) from each other. The Hereford was lighter ($P < .05$) than all breed groups except Angus and this difference approached significance ($P < .10$).

Large parental breed differences ($P < .01$) were observed for 200-day calf weight with Hereford significantly lighter (431 lb) than all other breed groups and Simmental heavier (575 lb) but Simmental was not different ($P > .05$) from Braunvieh, Gelbvieh and Pinzgauer.

Females of All Ages. Means are presented by breed group in Table 13 along with approximate difference between means, of parental breeds and between means of all breed groups, required for significance.

Large differences ($P < .01$) were observed among parental breeds for pregnant percentage with Limousin lowest (74.8%) and Red Poll highest (86.6%). The Limousin and Hereford did not differ ($P > .05$) from each other and neither did the Red Poll, Braunvieh, Angus, Simmental, Charolais, Gelbvieh and Pinzgauer. Large differences ($P < .01$) were observed among parental breeds in calf crop born

percentage. The Limousin was lowest (73.4%) and the Pinzgauer was highest (83.7%). Again, the Limousin and Hereford did not differ (P > .05) from each other and neither did the Red Poll, Braunvieh, Angus, Simmental, Charolais, Gelbvieh and Pinzgauer. Large differences (P < .01) were observed among parental breeds for calf crop weaned percentage with Limousin lowest (66.0%) and Red Poll highest (76.2%). The Limousin, Hereford and Simmental did not differ (P > .05) from each other and neither did the Red Poll, Braunvieh, Angus, Charolais, Gelbvieh and Pinzgauer. Differences between calf crop weaned percentage and calf crop born percentage reflect death losses, including dead at birth and from birth to weaning. The mean difference was 8.0% with Simmental greatest (10.8%) and Red Poll smallest (5.1%).

Large differences (P < .01) were observed among parental breeds in 200-day calf weight per female exposed to breeding with Hereford lowest (280 lb) and Gelbvieh highest (413 lb). The Hereford was lowest (P < .05) of all breed groups except Limousin and this difference approached significance (P < .10). The Gelbvieh did not differ (P > .05) from Pinzgauer, Braunvieh and Charolais.

Large differences (P < .01) were observed among parental breeds in 200-day calf weight with Hereford lightest (407 lb) and Simmental and Gelbvieh heaviest (544 lb). The Hereford was lighter (P < .05) than all parental breeds and the Gelbvieh and Simmental were heavier (P < .05) than all parental breeds except Braunvieh.

General. Differences among parental breeds were greater in the correlated traits of pregnant percentage, calf crop born percentage, calf crop weaned percentage and 200-day calf weight per female exposed in two yr old females than in females > five yr old or in females of all ages. The large differences among parental breed two yr old females in pregnant percentage is largely accounted for by differences in parental breed means in measures of puberty (Table 7 and 11). Even though of lesser magnitude than in two yr old females, large differences (P < .05) were observed among parental breed females of all ages in pregnant percentage, calf crop born percentage, calf crop weaned percentage, and in 200-day calf weight per female exposed. Thus, the handicap of reduced pregnancy rate as yearlings is reflected in a reduced pregnancy rate when averaged over all ages of females.

Differences Among Parental Breeds for Weight, Height and Condition Score

Data on one yr old females included in this report were collected 1) 168 days postweaning, 2) end of breeding season (452 days) and 3) when palpated for pregnancy (522 days). Data on 2-, 6- and from 2- through 7 or more yr old females included in this report were collected: 1) in February (about 2 mo before calving), 2) in June (before start of breeding season) and 3) in October (when palpated for pregnancy). Thus, the results presented in Tables 14, 15, 16, and 17 reflect the mean of observations made on these three data collection schedules within a year. Approximate differences between means, of parental breeds and between means of all breed groups, required for significance are presented in Tables 14, 15, 16, and 17. Cows that failed to wean a calf in a given year were excluded from the data set for that year.

Differences among parental breeds include additive direct genetic effects and additive maternal genetic effects ($G^I + G^m$).

One Year Old Females. Breed group means are provided in Table 14 for actual weight, weight adjusted to a common condition score, hip height and condition score. Large differences were observed among breed groups for all traits evaluated.

Two Year Old Females. Breed group means are provided in Table 15 for actual weight, weight adjusted to a common condition score, hip height and condition score. Similarly, large differences were observed among breed groups for all traits evaluated. The magnitude of difference in weight between specific breed groups was reduced considerably as a result of adjusting weight to a common condition score.

Six Year Old Females. Breed group means are provided in Table 16 for actual weight, weight adjusted to a common condition score, hip height and condition score. Again, large differences were observed among breed groups for all traits evaluated. Similarly, the magnitude of difference in weight was reduced considerably as a result of adjusting to a common condition score.

Two Through Seven or More Year Old Females. Breed group means are provided in Table 17 for actual weight, weight adjusted to a common condition score, hip height and condition score. Large differences were observed among breed groups for all traits evaluated.

Differences Among Parental Breeds in Milk Yield

Milk yield data were recorded using the weigh/nurse/weigh procedure for the 12 breed groups at intervals of five weeks when calf age averaged 8, 13 and 18 weeks (Table 18). Data were recorded in early June (before start of breeding season), mid-July (mid-breeding season) and in late August. Mean birth date was April 11 and calves were weaned in early September. Data were collected in 1990 and 1991. The intent was to sample the same 26 cow-calf pairs in each of the 12 breed groups three times during a season. However, in 1990 a problem was detected with the scale at one of the data collection sites after the mid-July collection. Thus, data from the mid-July collection at this site were eliminated and included the Hereford, Angus, Red Poll and composite MARC III breed groups for one collection time in one year. Thus, in 1990 data were collected only two times on these four breed groups. Also, cow or calf illness and (or) death reduced the number of observations to 1,686 from a potential of 1,872 (i.e., 26 cow-calf pairs of 12 breed groups for 3 collections in each of 2 years).

The day before collection, cows and calves were rounded up about 4:00 p.m., and penned until 6:00 p.m., at which time calves were separated from cows until 6:00 a.m., the following day when data collection started. Data were collected simultaneously at three sites in 1990 and at two sites in 1991. Even though all breed groups were not together they all had access to the same grasses at similar stages of maturity in adjacent pastures. Every effort was made to standardize experimental protocols among the sites. Calves were weighed to the nearest lb with an electronic scale immediately before and immediately after nursing for a period of 15-20 minutes. Calves were made to stand and to move for several minutes prior to first weighing to encourage voiding of urine and feces. Breed groups were separated at each weigh/nurse/weigh cycle in order to avoid the possibility of cross fostering among breed groups. In a few cases where cross fostering was detected within a

breed group, the data were eliminated. Care was taken to allow calves to receive all available milk produced by their dam but not left standing with dam for an extended period after completion of nursing. Calf weights were adjusted to 200 days but not adjusted for sex or age of dam.

Large differences were observed among parental breeds in 12 hr milk production (Table 18). Hereford was lowest ($P < .05$) and Braunvieh produced significantly more than all breed groups except Simmental where the difference approached significance. Parental breeds ranked similarly for 12-hr milk production and 200-day weight of progeny. The correlation among breed group means for 12-hr milk production with 200-day weight of progeny was .91.

Differences among parental breeds in 200-day weight adjusted by regression to a common estimated milk production are expected to reflect primarily differences in additive direct genetic effects (G^i) for 200-day weight. For 200-day weight adjusted to a common milk yield, Red Poll, Hereford, Angus and Limousin did not differ ($P > .05$) from each other and all were significantly lighter than Braunvieh, Pinzgauer, Gelbvieh, Simmental and Charolais which did not differ ($P > .05$) from each other (Table 18).

Genetic and Phenotypic Variation. Estimates of heritability (h^2) and their standard errors and phenotypic standard deviations (σ_p) were computed separately for purebreeds combined and for composite populations combined for all traits evaluated. These values are presented in tables with breed group means for the traits analyzed. Estimates of h^2 were computed using the sire within breed component of variance. Phenotypic standard deviations were computed by extracting the square root of the sum of the between and within sire components of variance. Generally, the differences between purebreeds combined and composite populations combined were small and were not consistent for estimates of both of h^2 and σ_p. There was no tendency for h^2's or σ_p to be greater for composite populations combined than for contributing purebreeds combined. Thus, greater genetic and phenotypic variation expected for composite populations combined than for purebreeds combined was not observed.

For greater detail see:

1. Keith E. Gregory, L. V. Cundiff, and R. M. Koch. 1991. Breed effects and heterosis in advanced generations of composite populations for preweaning traits of beef cattle. J. Anim. Sci. 69:947.
2. Keith E. Gregory, L. V. Cundiff, and R. M. Koch. 1991. Breed effects and heterosis in advanced generations of composite populations for growth traits in both sexes of beef cattle. J. Anim. Sci. 69:3202.
3. Keith E. Gregory, D. D. Lunstra, L. V. Cundiff, and R. M. Koch. 1991. Breed effects and heterosis in advanced generations of composite populations for puberty and scrotal traits of beef cattle. J. Anim. Sci. 69:2795.
4. Keith E. Gregory, L. V. Cundiff, and R. M. Koch. 1991. Breed effects and heterosis in advanced generations of composite populations for birth weight, birth date, dystocia, and survival as traits of dam in beef cattle. J. Anim. Sci. 69:3574.
5. Keith E. Gregory, L. V. Cundiff, and R. M. Koch. 1992. Breed effects and heterosis in advanced generations of composite populations for reproduction and maternal traits of beef cattle. J. Anim. Sci. 70:656.
6. Keith E. Gregory, L. V. Cundiff, and R. M. Koch. 1992. Breed effects and heterosis in advanced generations of composite populations on actual weight, adjusted weight, hip height, and condition score of beef cows. J. Anim. Sci. 70:1742.
7. Keith E. Gregory, L. V. Cundiff, and R. M. Koch. 1992. Effects of breed and retained heterosis on milk yield and 200-day weight in advanced generations of composite populations of beef cattle. J. Anim. Sci. 70:2366.

Table 1—Number of sires used and individuals born by birth year and breed group

Breed group	Number sires	Number indiv. born	Year of birth													
			1978	1979	1980	1981	1982	1983	1984	1985	1986	1987	1988	1989	1990	1991
Red Poll	51	1,322	47	129	109	114	110	109	109	88	80	84	84	87	87	85
Hereford	68	1,491	142	114	101	118	116	109	113	93	100	104	104	102	102	73
Angus	78	2,076	168	167	227	234	216	225	225	98	85	86	86	84	88	87
Limousin	56	1,478	86	127	117	115	117	121	107	99	106	98	105	96	104	80
Braunvieh	58	1,384	105	107	114	112	115	117	114	95	84	81	85	84	86	85
Pinzgauer	37	816					17	72	115	134	78	75	74	76	86	89
Gelbvieh	51	1,214	19	26	50	93	137	163	116	89	90	89	86	85	84	87
Simmental	67	1,410	145	117	111	110	116	113	111	90	88	80	82	82	84	81
Charolais	57	1,421	90	101	118	104	116	108	117	97	99	96	100	90	94	91
MARC I-F_1	20	583	33	87	141	112	107	103								
MARC I-F_2	24	1,081				38	74	121	147	132	145	121	117	100	86	
MARC I-F_3	45	806							41	65	128	116	122	107	108	119
MARC I-F_4	24	401										37	62	84	105	113
MARC II-F_1	17	730	143	198	183	132	74									
MARC II-F_2	28	1,328			48	100	181	223	199	117	110	105	98	82	65	
MARC II-F_3	42	974						42	99	174	115	116	107	105	103	113
MARC II-F_4	25	533									47	74	77	99	112	124
MARC III-F_1	15	556			115	108	118	113	102							
MARC III-F_2	24	925					42	70	129	174	144	112	100	85	69	
MARC III-F_3	31	694								38	73	119	132	118	97	117
MARC III-F_4	14	307											29	62	93	123

Table 2—Age of dam adjustment factors to a mature basis by sex and breed group

Breed Group	Age of Dam (yr)	Females			Intact Males		
		Birth Wt (lb)	Preweaning ADG (lb)	Postweaning ADG (lb)	Birth Wt (lb)	Preweaning ADG (lb)	Postweaning ADG (lb)
Red Poll	2	8.1129	0.1742	-0.1014	10.7804	0.2050	-0.0397
	3	3.8360	0.1257	-0.046	5.6658	0.1213	0.0044
	4	-2.8439	0.0132	-0.0088	1.1464	0.0243	0.0507
Hereford	2	8.4215	0.1962	-0.0728	10.0750	0.2712	-0.0220
	3	3.9903	0.1168	-0.0463	7.0547	0.1830	0.0331
	4	2.5794	0.0639	-0.0022	2.9541	0.0860	0.0353
Angus	2	5.8642	0.2116	-0.0705	8.2011	0.2778	-0.0198
	3	2.4912	0.1411	-0.0220	3.3289	0.1653	0.0309
	4	0.0000	0.0705	-0.0132	0.7275	0.0661	0.0000
Limousin	2	10.6261	0.2249	0.0309	12.3457	0.1962	-0.1345
	3	5.4894	0.1675	0.0331	7.6279	0.1367	0.0110
	4	0.5732	0.0926	0.0220	2.4691	0.0573	0.0044
Braunvieh	2	9.4136	0.2513	-0.0132	7.5617	0.2337	-0.0397
	3	5.9965	0.1587	0.0265	5.2469	0.1279	-0.0044
	4	0.7055	0.0353	0.0022	1.4330	0.0375	0.0353
Pinzgauer	2	15.9171	0.3571	-0.0044	22.5750	0.4563	0.0309
	3	6.3933	0.1918	0.0309	9.3474	0.2910	0.0705
	4	3.1746	0.0397	0.0507	4.7619	0.1235	0.0705
Gelbvieh	2	13.3818	0.2579	-0.0265	11.0009	0.2998	-0.0309
	3	8.4215	0.1565	0.0220	4.2328	0.2160	-0.0088
	4	3.3289	0.0816	0.0265	-1.2566	0.0772	0.0287
Simmental	2	12.5882	0.1918	-0.0772	13.3157	0.3175	-0.0309
	3	7.5838	0.1036	-0.0066	6.5035	0.2028	-0.0022
	4	0.0441	0.0044	-0.0044	0.3968	0.0904	0.0375
Charolais	2	13.4700	0.2756	-0.0176	18.6728	0.3175	-0.0088
	3	4.0564	0.2160	-0.0287	7.0547	0.2513	0.0000
	4	1.1464	0.0617	-0.0485	2.0062	0.0948	0.0375
MARC I	2	12.4339	0.2116	-0.0265	12.0811	0.2535	-0.0287
	3	6.7019	0.1455	-0.0132	6.6799	0.1477	0.0353
	4	2.4030	0.0353	-0.0309	1.4550	0.0287	0.0309
MARC II	2	7.4515	0.2072	-0.0750	10.0529	0.2690	-0.0485
	3	0.5071	0.1190	-0.0595	0.6393	0.1764	0.0176
	4	-0.5291	-0.0132	-0.0551	-0.4850	0.0441	-0.0022
MARC III	2	13.3818	0.2006	-0.0617	12.0811	0.2734	-0.0353
	3	3.5714	0.1279	-0.0683	1.2787	0.1455	-0.0287
	4	1.8519	-0.0110	-0.0132	0.6614	0.0683	0.0220

Table 3—Breed group means for growth traits - females

Breed group		Number	Birth weight (lb)	200-day weight (lb)	368-day weight (lb)	368-day height (in)	368-day condition score[a]
Overall mean		7,785	87.8	490	750	47.2	5.2
Red Poll		521	80.7	445	675	45.7	5.0
Hereford		537	76.5	392	631	44.1	5.6
Angus		780	71.9	423	681	44.5	5.9
Limousin		526	82.9	450	692	47.2	3.7
Braunvieh		490	94.8	525	776	48.8	4.6
Pinzgauer		282	97.7	520	776	48.4	4.6
Gelbvieh		439	92.2	536	785	48.8	4.8
Simmental		506	91.3	527	787	48.8	4.6
Charolais		538	95.0	512	787	48.8	4.7
h^2[d]			.40±.05	.32±.04	.38±.05	.39±.05	.43±.05
σ_p[e]			9.7	44.0	63.8	1.3	.9
D.05[b]			2.6	11.5	17.4	.4	.2
MARC I	F_1	239	92.2	514	794	48.0	5.5
	F_2	430	92.4	512	785	48.0	5.2
	F_3&$_4$	304	93.0	514	789	48.4	5.1
MARC II	F_1	331	85.6	518	778	47.2	6.1
	F_2	536	88.4	494	765	46.8	5.8
	F_3&$_4$	436	87.3	500	770	47.2	5.7
MARC III	F_1	243	85.6	476	741	46.4	5.7
	F_2	394	85.6	478	743	46.1	5.8
	F_3&$_4$	253	86.2	472	736	46.1	5.6
h^2[d]			.34±.05	.27±.05	.31±.05	.41±.06	.31±.05
σ_p[e]			10.9	45.5	66.2	1.4	.9
D.05[c]			3.3	14.1	21.4	.5	.3

[a] 9 = highest, 1 = lowest.

[b] D.05 is the approximate difference between means of parental breeds required for significance.

[c] D.05 is the approximate difference between means of all breed groups required for significance.

[d] h^2 = heritability.

[e] σ_p = phenotypic standard deviation.

Table 4—Breed group means for growth traits - males

Breed group		Number	Birth weight (lb)	200-day weight (lb)	368-day weight (lb)	368-day height (in)	368-day condition score[a]	368-day muscling score[a]
Overall mean		7,055	94.2	523	986	48.8	4.9	5.3
Red Poll		419	86.2	487	902	47.6	5.0	4.0
Hereford		489	81.8	419	842	45.7	5.6	4.6
Angus		754	77.0	450	882	46.1	5.6	5.1
Limousin		477	90.0	481	911	49.2	3.3	6.9
Braunvieh		454	102.1	556	1,019	50.4	4.2	5.0
Pinzgauer		222	108.0	560	1,039	49.6	4.8	4.8
Gelbvieh		377	98.1	571	1,036	50.4	4.3	5.6
Simmental		451	98.1	562	1,052	50.8	4.6	5.7
Charolais		421	102.3	542	1,034	50.4	4.1	5.9
h^2[d]			.50±.06	.27±.04.	43±.05	.39±.05	.38±.05	.37±.05
σ_p[e]			10.6	46.7	76.2	1.3	.8	.7
D.05[b]			3.1	12.8	22.0	.4	.2	.2
MARC I	F_1	242	95.7	536	1,014	49.6	4.7	5.8
	F_2	448	97.7	538	1,008	49.6	4.5	5.7
	$F_{3\&4}$	247	97.9	534	990	49.6	4.4	5.6
MARC II	F_1	344	91.9	567	1,028	49.6	5.5	5.2
	F_2	555	95.0	529	1,008	48.8	5.6	5.3
	$F_{3\&4}$	403	94.4	538	1,025	49.2	5.5	5.2
MARC III	F_1	237	92.4	516	975	48.4	5.7	4.9
	F_2	381	92.8	518	986	48.0	5.6	4.7
	$F_{3\&4}$	134	93.5	512	988	48.0	5.4	4.8
h^2[d]			.34±.06	.23±.05	.26±.05	.47±.06	.29±.05	.30±.05
σ_p[e]			11.9	49.3	82.6	1.4	.8	.6
D.05[c]			3.7	15.4	26.5	.5	.3	.2

[a] 9 = highest, 1 = lowest.
[b] D.05 is the approximate difference between means of parental breeds required for significance.
[c] D.05 is the approximate difference between means of all breed groups required for significance.
[d] h^2 = heritability.
[e] σ_p = phenotypic standard deviation

Table 5—Breed group means for puberty traits of females and scrotal circumference of males

Breed group		Number	Puberty 368 days (%)	Puberty 410 days (%)	Puberty 452 days[b] (%)	Adjusted age[c] (days)	Adjusted weight[c] (lb)	Number	Scrotal circumference[d] (cm)
Overall mean		6,034	72.4	79.8	94.8	370	736	6,649	32.8
Red Poll		450	83.7	88.6	97.4	359	650	410	33.1
Hereford		427	31.7	39.9	82.8	411	695	472	30.3
Angus		670	46.1	57.4	93.3	393	697	738	32.1
Limousin		403	36.1	44.0	79.3	408	743	464	29.0
Braunvieh		359	89.7	94.2	100.0	350	732	444	33.7
Pinzgauer		246	85.8	92.1	96.6	360	739	215	33.0
Gelbvieh		330	86.3	92.9	99.1	353	745	366	34.1
Simmental		358	77.4	86.8	98.0	363	758	437	33.7
Charolais		406	50.7	60.6	86.5	391	814	406	32.2
Parental breed mean			65.3	72.9	92.6	376	730		32.4
h^2[h]			.28±.05	.31±.05	.32±.05	.33±.06	.47±.06		.54±.06
σ_p[i]		40.8		38.9	25.1	28.9	65.3		2.5
D.05[e]			11.0	10.0	6.2	8.1	20.7		.7
MARC I	F_1	182	78.0	85.5	99.2	366	767	240	32.5
	F_2	332	76.3	85.8	98.7	366	765	405	32.7
	F_3&$_4$[g]	190	73.4	83.0	94.6	367	763	201	33.0
MARC II	F_1	274	89.8	95.2	97.6	360	745	340	34.1
	F_2	410	82.6	89.3	97.4	361	738	502	33.6
	F_3&$_4$[g]	239	80.3	86.9	95.3	360	739	344	33.8
h^2[h]			22±.05	.13±.05	.09±.04	.27±.06	.37±.07		.45±.07
σ_p			36.6	30.6	15.9	25.4	66.7		2.5
MARC III	F_1	243	86.2	91.2	100.0	361	710	233	33.6
	F_2	358	77.6	84.0	95.1	368	723	330	32.8
	F_3&$_4$[g]	157	71.8	79.0	94.4	370	723	102	32.8
D.05[f]			13.5	12.4	7.7	10.0	25.6		.9

[a] 410 days = start of breeding season.
[b] 452 days = end of breeding season.
[c] Adjusted to 100% puberty basis.
[d] Adjusted to a common age.
[e] D.05 is the approximate difference between means of parental breeds required for significance.
[f] D.05 is the approximate difference between means of all breed groups required for significance.
[g] F_4 generation for scrotal circumference only.
[h] h^2 = heritability.
[i] σ_p = phenotypic standard deviation.

Table 6—Correlation coefficients among parental breed means for puberty traits of females with scrotal circumference of males

Female Puberty Traits	Scrotal Circumference (cm)
% Puberty at 368 days	.88**
% Puberty at 410 days	.91**
% Puberty at 452 days	.95**
Age at Puberty - days	-.91**
Age at Puberty - Adj. for 368 d wt.	-.91**

** P < .01.

Table 7—Correlation coefficients among parental breed means for puberty traits with pregnancy percentage in yearling females

Female Puberty Traits	Pregnancy (%)
% Puberty at 368 days	.87**
% Puberty at 410 days	.89**
% Puberty at 452 days	.97**
Age at Puberty - days	-.89**
Age at Puberty - Adj. for 368 d wt.	-.88**

** P < .01.

Table 8—Breed group means for birth and survival traits as traits of dam - two years old

Breed group		Number	Birth weight (lb)	Birth date (Julian)	Calving difficulty[d] (%)	Survival		
						Birth (%)	72 hr (%)	Weaning (%)
Overall mean		4,140	83.8	78	52.5	95.5	89.0	81.0
Red Poll		268	76.7	74	54.0	95.7	90.6	85.4
Hereford		242	74.5	84	49.1	93.2	86.2	74.3
Angus		433	69.4	75	31.8	93.0	86.2	81.4
Limousin		210	79.4	88	40.6	96.0	85.1	76.2
Braunvieh		287	93.3	80	73.8	98.2	90.8	81.0
Pinzgauer		250	92.4	74	62.1	95.2	88.9	82.3
Gelbvieh		321	88.4	79	60.5	93.6	88.1	79.2
Simmental		312	85.3	79	52.5	97.9	91.2	80.9
Charolais		261	88.4	78	48.6	98.0	92.8	82.8
Parental breed mean			83.1	79	52.6	95.6	88.9	80.4
h^2[e]			19±.05	.08±.05	.14±.05	-	.03±.04	.06±.05
σ_p[f]			10.5	11.5	46.1	20.3	31.1	38.5
D.05[a]			3.3	3.3	13.2	5.1	8.0	9.9
MARC I	F_1[b]	167	88.4	77	55.5	95.4	89.8	82.1
	F_2&F_3[b]	308	90.0	79	56.6	96.8	90.7	82.9
MARC II	F_1[b]	232	83.8	76	50.6	94.6	86.8	78.4
	F_2&F_3[b]	393	85.8	75	57.1	95.1	87.8	83.1
MARC III	F_1[b]	192	80.9	72	46.2	95.1	89.5	84.4
	F_2&F_3[b]	264	81.4	76	48.4	95.2	90.1	81.2
h^2[e]			.21±.07	.18±.07	.09±.06	-	-	-
σ_p[f]			11.3	11.5	47.7	21.0	30.1	37.5
D.05[c]		3.7	3.7	14.9	5.8	9.1	11.2	

[a] D.05 is the approximate difference between means of parental breeds required for significance.
[b] F_1 generation females producing F_2 generation progeny and combined F_2 & F_3 generation females producing F_3 & F_4 generation progeny.
[c] D.05 is the approximate difference between means of all breed groups required for significance.
[d] Percentage requiring assistance.
[e] h^2 = heritability.
[f] σ_p = phenotypic standard deviation.

Table 9—Breed group means for birth and survival traits as traits of dam – three or more years old

Breed group		Number	Birth weight (lb)	Gestation length (days)	Birth date (Julian)	Calving difficulty[d] (%)	Survival		
							Birth (%)	72 hr (%)	Weaning (%)
Overall mean		10,710	93.3	287	104	8.1	98.2	96.3	93.3
Red Poll		706	86.2	288	103	3.0	98.7	97.4	95.6
Hereford		818	81.1	288	107	5.1	97.6	95.7	93.9
Angus		1,133	76.3	283	99	.9	98.5	94.9	91.7
Limousin		871	88.6	289	109	7.2	98.3	96.6	93.2
Braunvieh		714	100.1	290	109	13.2	98.2	96.9	91.9
Pinzgauer		391	104.5	287	103	15.9	96.1	94.4	91.6
Gelbvieh		677	97.7	287	107	8.3	99.2	97.6	93.7
Simmental		671	97.0	287	106	14.4	97.0	93.4	88.1
Charolais		784	101.4	286	105	9.8	98.8	97.3	93.4
Parental breed mean			92.6	287	105	8.6	98.0	96.0	92.6
h^2[a]			.33±.05	.52±.06	.20±.04	.12±.03	.12±.03	.04±.03	-
σ_p[f]			10.8	4.4	9.1	26.8	12.0	18.5	26.2
D.05[a]			2.4	1.6	2.8	5.1	2.0	2.8	4.0
MARC I	F_1[b]	828	98.1	287	106	8.2	97.2	96.2	93.3
	F_2&F_3[b]	453	97.9	288	104	8.9	98.6	96.5	94.9
MARC II	F_1[b]	1,031	94.2	287	102	8.3	98.7	96.9	94.8
	F_2&F_3[b]	662	93.3	287	104	9.3	98.8	96.5	93.8
MARC III	F_1[b]	664	91.5	287	103	3.3	98.9	98.1	96.0
	F_2&F_3[b]	307	91.7	286	100	6.1	97.9	96.2	93.2
h^2[e]			.39±.06	.74±.09	.22±.05	-	-	-	-
σ_p[f]			11.9	4.6	8.2	25.1	12.9	17.2	22.1
D.05[c]			2.9	1.9	3.3	5.8	2.3	3.2	4.7

[a] D.05 is the approximate difference between means of parental breeds required for significance.

[b] F_1 generation females producing F_2 generation progeny and combined F_2 & F_3 generation females producing F_3 & F_4 generation progeny.

[c] D.05 is the approximate difference between means of all breed groups required for significance.

[d] Percentage requiring assistance.

[e] h^2 = heritability.

[f] σ_p = phenotypic standard deviation.

Table 10—Breed group means for birth and survival traits as traits of dam - all ages

Breed group		Number	Birth weight (lb)	Birth date (Julian)	Calving difficulty[d] (%)	Survival		
						Birth (%)	72 hr (%)	Weaning (%)
Overall mean		14,850	91.1	98	19.2	97.6	94.5	90.4
Red Poll		974	83.8	96	15.9	98.0	95.9	93.4
Hereford		1,060	79.4	101	16.5	96.6	93.3	89.2
Angus		1,566	74.7	93	8.8	97.2	92.8	89.3
Limousin		1,081	86.4	104	15.7	97.8	93.8	89.2
Braunvieh		1,001	98.6	102	28.5	98.3	95.5	89.3
Pinzgauer		641	101.9	96	27.4	96.0	92.9	89.0
Gelbvieh		998	95.2	100	21.5	97.9	95.3	90.4
Simmental		983	94.2	99	23.9	97.3	92.9	86.4
Charolais		1,045	98.1	98	19.5	98.7	96.3	90.9
Parental breed mean			90.2	99	19.7	97.5	94.3	89.7
h_2[e]			.22±.03	.10±.02	13.2±.02	.03±.01	.02±.01	.02±.01
σ_p[f]			11.0	14.3	33.2	15.6	23.3	30.2
D.05[e]			2.2	2.4	5.8	2.2	3.2	4.2
MARC I	F_1[b]	995	95.9	98	19.5	97.0	94.7	90.8
	F_2&F_3[b]	761	96.1	98	20.7	98.2	95.1	92.0
MARC II	F_1[b]	1,263	91.7	96	19.1	97.8	94.1	90.6
	F_2&F_3[b]	1,055	91.7	96	21.0	97.9	94.5	91.4
MARC III	F_1[b]	856	89.1	94	13.9	98.1	96.0	93.5
	F_2&F_3[b]	571	89.3	94	16.7	97.3	94.7	90.3
h_2[e]			.14±.03	.07±.02	.08±.02	-	-	-
σ_p[f]			12.4	13.8	33.6	15.6	21.6	27.3
D.05[c]			2.6	2.7	6.6	2.5	3.7	4.8

[e] D.05 is the approximate difference between means of parental breeds required for significance.

[b] F_1 generation females producing F_2 generation progeny and combined F_2 & F_3 generation females producing F_3 & F_4 generation progeny.

[c] D.05 is the approximate difference between means of all breed groups required for significance.

[d] Percentage requiring assistance.

[e] h_2 = heritability.

[f] σ_p = phenotypic standard deviation.

Table 11—Breed group means for reproduction and maternal traits - two years old

Breed group		Number	Pregnant (%)[a]	Calf crop born (%)[a]	Calf crop weaned (%)[e]	200-day calf wt/ female exp. (lb)[e]	200-day calf wt (lb)
Overall mean		6,535	77.3	74.4	60.6	284	463
Red Poll		436	81.0	77.0	66.2	286	432
Hereford		453	64.1	62.3	46.8	178	378
Angus		685	77.9	75.4	61.8	249	402
Limousin		474	54.7	53.0	41.8	179	427
Braunvieh		426	83.0	80.3	66.4	334	500
Pinzgauer		373	81.6	79.3	64.1	314	486
Gelbvieh		458	85.6	83.2	66.2	340	506
Simmental		477	82.4	81.2	66.0	331	498
Charolais		476	72.3	67.2	56.2	269	478
Parental breed mean			75.8	73.2	59.5	276	456
h^2[e]			.20±.04	.24±.04	.18±.04	.17±.04	.32±.07
σ_p			41.6	44.4	48.0	221	48.0
D.05[b]			10.5	11.4	11.6	54.0	11.5
MARC I	F_1[c]	230	78.2	75.1	62.1	305	487
	F_2&F_3[c]	551	86.3	83.5	69.0	336	486
MARC II	F_1[c]	331	73.9	71.3	56.7	269	472
	F_2&F_3[c]	714	74.8	71.8	59.1	288	484
MARC III	F_1[c]	250	82.2	79.4	66.9	308	460
	F_2&F_3[c]	501	81.6	74.7	60.5	275	451
h_2[e]			.24±.05	.27±.06	.13±.05	.14±.05	.19±.07
σ_p[f]			40.4	43.9	48.2	233	50.0
D.05[d]			11.9	13.0	13.2	61.5	13.0

[a] Based on females exposed to breeding; pregnant (%) determined by rectal palpation.

[b] D.05 is the approximate difference between means of parental breeds required for significance.

[c] F_1 generation females producing F_2 generation progeny and combined F_2 & F_3 females producing F_3 & F_4 generation progeny.

[d] D.05 is the approximate difference between means of all breed groups required for significance.

Table 12—Breed group means for reproduction and maternal traits -five or more years old

Breed group		Number	Pregnant (%)[a]	Calf crop born (%)[a]	Calf crop weaned (%)[a]	200-day calf wt/ female exp. (lb)[a]	200-day calf wt (lb)
Overall mean		7,920	90.8	87.4	83.0	438	528
Red Poll		607	89.6	86.1	84.2	411	489
Hereford		728	86.0	84.4	80.7	348	431
Angus		1,030	91.3	87.9	82.4	384	465
Limousin		736	87.8	87.0	82.6	406	491
Braunvieh		599	90.6	88.4	84.2	476	566
Pinzgauer		188	90.5	87.4	79.3	445	565
Gelbvieh		328	88.5	85.6	82.6	474	571
Simmental		516	87.8	84.8	78.8	453	575
Charolais		569	90.8	89.7	85.2	477	560
Parental breed mean			89.2	86.8	82.2	430	523
h^2[a]			.02±.02	.08±.04	.08±.04	.10±.04	.34±.06
σ_p[f]			31.5	39.2	42.1	219	51.4
D.05[b]			5.7	7.6	8.3	44.1	12.3
MARC I	F_1[c]	624	92.9	92.1	86.6	473	546
	F_2&F_3[c]	202	95.2	92.5	88.6	484	546
MARC II	F_1[c]	820	93.3	90.7	85.3	448	523
	F_2&F_3[c]	347	91.3	88.4	84.4	467	553
MARC III	F_1[c]	522	92.4	86.5	83.0	427	516
	F_2&F_3[c]	104	94.4	80.8	77.4	406	525
h^2[a]			.03±.03	.11±.04	.10±.04	.08±.04	.30±.07
σ_p[f]			26.0	31.8	36.2	204	57.9
D.05[d]			6.6	8.7	9.5	50.9	14.3

[a] Based on females exposed to breeding; pregnant (%) determined by rectal palpation.

[b] D.05 is the approximate difference between means of parental breeds required for significance.

[c] F_1 generation females producing F_2 generation progeny and combined F_2 & F_3 generation females producing F_3 & F_4 generation progeny.

[d] D.05 is the approximate difference between means of all breed groups required for significance.

Table 13—Breed group means for reproduction and maternal traits - all ages

Breed group		Number	Pregnant (%)[a]	Calf crop born (%)[a]	Calf crop weaned (%)[a]	200-day calf wt/ female exp. (lb)[a]	200-day calf wt (lb)
Overall mean		24,342	84.7	81.6	73.8	372	502
Red Poll		1,710	86.6	81.3	76.2	356	467
Hereford		1,835	78.9	76.3	68.2	280	407
Angus		2,763	84.6	81.0	72.6	320	439
Limousin		1,958	74.8	73.4	66.0	306	461
Braunvieh		1,696	85.0	82.4	73.9	400	539
Pinzgauer		1,066	86.3	83.7	74.8	401	534
Gelbvieh		1,365	85.2	83.2	75.5	413	544
Simmental		1,718	83.1	80.8	70.0	382	544
Charolais		1,804	83.2	80.8	73.7	387	522
Parental breed mean			83.1	80.3	72.3	361	495
h^2[e]			.06±.01	.09±.02	.07±.01	.07±.01	
σ_p[f]			36.3	41.7	45.	223	
D.05[b]			4.6	5.5	5.0	28.2	8.4
MARC I	F_1[c]	1,281	88.7	86.7	78.8	414	522
	F_2&F_3[c]	1,301	88.5	85.2	77.6	409	523
MARC II	F_1[c]	1,739	86.5	84.3	76.6	394	512
	F_2&F_3[c]	1,825	84.0	81.5	73.8	389	523
MARC III	F_1[c]	1,202	89.6	84.8	79.2	395	497
	F_2&F_3[c]	1,079	86.0	78.0	70.5	349	492
h_2[a]			.07±.02	.07±.02	.05±.02	.06±.02	
σ_p[f]			33.2	37.9	42.3	220	
D.05[d]			5.3	6.2	5.7	32.2	9.7

[a] Based on females exposed to breeding; pregnant (%) determined by rectal palpation.

[b] D.05 is the approximate difference between means of parental breeds required for significance.

[c] F_1 generation females producing F_2 generation progeny and combined F_2 & F_3 generation females producing F_3 & F_4 generation progeny.

[d] D.05 is the approximate difference between means of all breed groups required for significance.

Table 14—Breed group means for weight, height and condition score - one year old

Breed group	Number	Actual weight (lb)	Adjusted weight[a] (lb)	Height (in)	Condition score[b]
Overall mean	23,292	807	812	48.6	5.0
Red Poll	1,578	739	743	47.2	5.0
Hereford	1,575	712	694	45.7	5.7
Angus	2,211	743	730	46.1	5.6
Limousin	1,665	756	783	48.4	3.6
Braunvieh	1,488	842	862	50.0	4.6
Pinzgauer	957	831	847	49.6	4.6
Gelbvieh	1,254	851	864	50.0	4.8
Simmental	1,584	860	875	50.4	4.7
Charolais	1,665	866	880	50.0	4.7
h_2[e]		.65±.04	.66±.04	.65±.04	.44±.03
σ_p[f]		70.0	65.5	1.4	.8
D.05[c]		21.2	20.0	.4	.2
MARC I					
F_1,F_2 & F_3	2,973	853	853	49.6	5.2
MARC II					
F_1,F_2 & F_3	3,633	833	820	48.4	5.8
MARC III					
F_1,F_2 & F_3	2,709	803	792	47.6	5.6
h^2[e]		.36±.04	.39±.04	.53±.05	.31±.04
σ_p[f]		73.9	71.0	1.4	.8
D.05[d]		26.0	23.2	.5	.3

[e] Adjusted to a common condition score.
[b] 9 = highest, 1 = lowest.
[c] D.05 is the approximate difference between means of parental breeds required for significance.
[d] D.05 is the approximate difference between means of all breed groups required for significance.
[e] h^2 = heritability.
[f] σ_p = phenotypic standard deviation.

Table 15—Breed group means for weight, height and condition score - two years old

Breed group	Number	Actual weight (lb)	Adjusted weight[a] (lb)	Height (in)	Condition score[b]
Overall mean	13,002	1,024	1,029	52.3	5.3
Red Poll	924	906	930	49.9	5.0
Hereford	714	944	910	49.0	6.0
Angus	1,320	933	924	48.5	5.6
Limousin	723	999	1,038	51.8	4.3
Braunvieh	903	1,052	1,080	52.6	4.8
Pinzgauer	753	1,025	1,047	52.0	5.0
Gelbvieh	879	1,069	1,078	53.2	5.3
Simmental	972	1,085	1,098	53.7	5.2
Charolais	945	1,146	1,146	53.3	5.5
h^{2e}		.82±.06	.76±.05	.70±.05	.39±.04
σ_p[f]		91.0	81.4	1.4	.9
D.05[c]		30.6	26.9	.4	.2
MARC I					
F_1,F_2 & F_3	1,518	1,069	1,071	52.1	5.4
MARC II					
F_1,F_2 & F_3	1,797	1,041	1,019	51.4	6.0
MARC III					
F_1,F_2 & F_3	1,554	1,019	1,005	50.5	5.8
h^{2e}		.57±.06	.56±.06	.57±.06	.28±.04
σ_p[f]		97.4	89.5	1.5	.8
D.05[d]		38.6	34.0	.6	.3

[e] Adjusted to a common condition score.

[b] 9 = highest, 1 = lowest.

[c] D.05 is the approximate difference between means of parental breeds required for significance.

[d] D.05 is the approximate difference between means of all breed groups required for significance.

[e] h^2 = heritability.

[f] σ_p = phenotypic standard deviation.

Table 16—Breed group means for weight, height and condition score - six years old

Breed group	Number	Actual weight (lb)	Adjusted weight[a] (lb)	Height (in)	Condition score[b]
Overall mean	4,455	1,301	1,287	52.5	5.7
Red Poll	339	1,200	1,188	51.2	5.8
Hereford	396	1,257	1,173	50.4	6.9
Angus	585	1,230	1,184	50.0	6.4
Limousin	390	1,261	1,294	52.8	4.5
Braunvieh	318	1,318	1,334	53.4	5.2
Pinzgauer	90	1,274	1,290	52.8	5.2
Gelbvieh	201	1,349	1,352	53.9	5.5
Simmental	273	1,341	1,349	54.3	5.3
Charolais	315	1,438	1,431	54.3	5.7
h^2[e]		1.00±.09	.99±.09	.83±.08	.56±.07
σ_p[f]		106.1	95.3	1.4	1.0
D.05[c]		38.1	33.1	.5	.3
MARC I F_1,F_2 & F_3	492	1,358	1,341	52.8	5.9
MARC II F_1,F_2 & F_3	672	1,290	1,263	52.4	6.1
MARC III F_1,F_2 & F_3	384	1,296	1,248	51.6	6.4
h^2[e]		.87±.12	.84±.12	.62±.10	.40±.08
σ_p[f]		120.8	111.7	1.5	.9
D.05[d]		40.4	36.2	.6	.4

[a] Adjusted to a common condition score.
[b] 9 = highest, 1 = lowest.
[c] D.05 is the approximate difference between means of parental breeds required for significance.
[d] D.05 is the approximate difference between means of all breed groups required for significance.
[e] h^2 = heritability.
[f] σ_p = phenotypic standard deviation.

Table 17—Breed group means for weight, height and condition score - two through seven or more years old

Breed group	Number	Actual weight (lb)	Adjusted weight[a] (lb)	Height (in)	Condition score[b]
Overall mean	49,251	1,210	1,208	52.4	5.5
h^2					
σ_p					
Red Poll	3,447	1,098	1,105	50.8	5.4
Hereford	3,516	1,149	1,091	50.0	6.5
Angus	5,022	1,118	1,094	49.6	6.0
Limousin	3,822	1,175	1,213	52.4	4.4
Braunvieh	3,393	1,241	1,266	53.5	4.9
Pinzgauer	2,184	1,197	1,217	52.8	5.1
Gelbvieh	2,706	1,257	1,266	53.9	5.3
Simmental	3,258	1,261	1,272	54.3	5.3
Charolais	3,618	1,352	1,349	53.9	5.5
h^2[e]		.68±.04	.63±.04	.66±.04	.34±.02
σ_p[f]		105	94	1.4	.9
D.05[c]		27.8	24.5	.4	.18
MARC I					
F_1, F_2 & F_3	5,820	1,270	1,263	52.8	5.7
MARC II					
F_1, F_2 & F_3	7,389	1,217	1,193	52.0	6.0
MARC III					
F_1, F_2 & F_3	5,076	1,202	1,171	51.2	6.0
h^2[e]		.60±.05	.59±.05	.54±.05	.27±.03
σ_p[f]		114	105	1.6	.9
D.05[d]		26.7	23.6	.4	.17

[a] Adjusted to a common condition score.
[b] 9 = highest, 1 = lowest.
[c] D.05 is the approximate difference between means of parental breeds required for significance.
[d] D.05 is the approximate difference between means of all breed groups required for significance.
[e] h^2 = heritability.
[f] σ_p = phenotypic standard deviation.

Table 18—Breed group means for 12-hr milk yield, estimated 200-day milk yield and 200-day weight

Breed group	Number observations	12-hr milk yield (lb)	No. cows	Estimated 200-day milk yield (lb)	200-day weight of progeny (lb)	Adjusted 200-day weight of progeny[a] (lb)
Overall mean	1,686	11.5	595	4,604	503	494
Red Poll	118	11.9	46	4,774	478	463
Hereford	122	6.7	45	2,774	408	459
Angus	125	9.3	48	3,735	454	472
Limousin	149	10.2	50	4,114	456	459
Braunvieh	147	14.2	52	5,680	558	520
Pinzgauer	156	12.9	52	5,173	531	505
Gelbvieh	150	12.7	51	5,120	545	520
Simmental	151	13.1	51	5,283	545	516
Charolais	146	10.5	50	4,212	518	518
h^2[e]		.62±.11	.39±.23	.10±.23		
σ_p[f]		2.6		816	44.0	
D.05[b]		1.3		531	28.4	24.2
MARC I[c]	155	12.5	52	5,034	527	505
MARC II[c]	147	11.7	50	4,732	529	514
MARC III[c]	120	11.6	48	4,613	494	481
h^2[e]		.40±.16		-	.67	
σ_p[f]		2.6		820	43.7	
D.05[d]		1.3		560	30.0	25.6

[e] Adjusted to a common estimated milk yield.
[b] D.05 is the approximate difference between means of parental breeds required for significance.
[c] F_2 generation females nursing F_3 generation progeny.
[d] D.05 is the approximate difference between means of all breed groups required for significance.
[e] h^2 = heritability.
[f] σ_p = phenotypic standard deviation.

Estimates of Genetic and Phenotypic Parameters of Pelvic Measures, Weight, Height, Calf Birth Weight, and Dystocia in Beef Cattle

Keith E. Gregory, Larry V. Cundiff, and Robert M. Koch[1]

Introduction

Based on requirements for assistance at first parturition as two-year-olds, experimental results document the importance of dystocia in major breeds of *Bos taurus* cattle. In addition to the greater labor and managerial requirements associated with dystocia (calving difficulty), experimental results show that dystocia results in reduced perinatal calf survival and reduced conception rate in females in the subsequent breeding season when dystocia is experienced. There is not agreement on the value of pelvic measures as a predictor of dystocia at first parturition. Information is limited on the genetic relationship between pelvic measures and other factors that may be genetically associated with dystocia. Selection criteria and procedures that have high predictive value for dystocia and can be evaluated prior to an age of one year when selection decisions are normally made are needed to optimally combine information on a series of bioeconomic traits to increase selection response for reducing dystocia without loss in postnatal growth rate. Because most of the selection opportunity in cattle is among males, selection criteria among males must have high predictive value in their female progeny. The purpose of this study was to provide estimates of genetic and phenotypic parameters on a series of bioeconomic traits evaluated at, or prior to, one year of age as a basis for developing selection criteria and procedures that may result in reduced dystocia while maintaining rate of postnatal gain.

Procedure

Populations. Breed groups included in this study were nine purebreeds [e.g., Red Poll (R), Hereford (H), Angus (A), Limousin (L), Braunvieh (B), Pinzgauer (P), Gelbvieh (G), Simmental (S) and Charolais (c)] and three composite populations to which the nine purebreeds contributed, (MARC I = 1/4 B, 1/4 C, 1/4 L, 1/8 H, 1/8 A; MARC II = 1/4 G, 1/4 S, 1/4 H, 1/4 A and MARC III = 1/4 R, 1/4 P, 1/4 H, 1/4 A). Data were collected on F_1, F_2, F_3 and F_4 generations from composite MARC I; F_2, F_3 and F_4 generations from composite MARC II and F_1, F_2, F_3 and F_4 generations from composite MARC III. The cattle contributing data for this study were in the Germplasm Utilization Project and were born in the years 1983 through 1990.

Data Collection. Calves were weighed at birth, at weaning and 140 and 168 days postweaning. Height was measured at 168 days postweaning in both sexes. Pelvic measures (width and height) were recorded 140 days postweaning in both intact males and females at an average age of 320 days. Pelvic measures were taken by two or three experienced technicians in each year. From 1983 through 1985 measures were taken by the Krautman-Litton Pelvic Meter[2] and since 1986 were taken by the Rice Pelvimeter[3].

Calving difficulty was subjectively evaluated using descriptive scores; i.e., 1 = no difficulty, 2 = little difficulty by hand, 3 = little difficulty with calf jack, 4 = slight difficulty with a calf jack, 5 = moderate difficulty with calf jack, 6 = major difficulty with calf jack, 7 = caesarean birth and 8 = abnormal presentation. Percentage calving difficulty was analyzed (scores and 2 = 0; scores 3, 4, 5, 6 and 7 = 1; and scores of 8 were excluded from analyses). Scores of 8 also were excluded from analysis of calving difficulty score.

Analysis of Data. The data were analyzed by least-squares mixed model procedures. The models used included the fixed effects of breed group, year of birth, age of dam with date of birth included as a covariate to adjust to a common age. Sire within breed group was treated as a random effect. More information on specific analyses of these data is provided in the section on Results and is reflected by Tables 1 through 12.

Studentized Range as described by Snedecor and Cochran (1980, p. 234) was computed to obtain approximations of differences required for significance among breed group means for the traits evaluated (Tables 1, 2, 3 and 4).

Separate analyses were conducted for the nine purebreeds and the combined generations of the three composite populations. There was no difference between the purebreeds and the three composite populations in either phenotypic or genetic variation for the traits analyzed. Thus, they were treated as 12 breed groups in each analysis.

Results

Heritability (h^2), genetic correlations (rg), and phenotypic correlations (rp) among pelvic height, pelvic width, pelvic area, 368-day weight and 368-day height were estimated on 5,715 female progeny by 552 sires and 4,531 male progeny by 503 sires (Tables 1 and 2). Two analyses were conducted for each sex, i.e., (1) all traits included (Tables 5 and 7) and (2) pelvic measures adjusted by regression to a common weight and height (Tables 6 and 8). Genetic (co)variances were estimated from the sire within breed group variancecomponent for 12 breed groups representing nine purebred and three composite populations. Among females that produced calves as two-yr-olds (2,942 females by 438 sires), (Tables 3 and 4), the traits of calf birth weight, calving difficulty score (1 through 7) and calving difficulty percentage (0 or 1) were added and four separate analyses were conducted: (1) all calves with sex included in the model (Table 9); (2) traits adjusted by regression to a common birth weight (Table 10); (3) females producing female calves (Table 11); and (4) females producing male calves (Table 12).

The h^2's for pelvic measures were greater in males than in females (Tables 5 and 7). The h^2's for pelvic measures were not greatly reduced as a result of adjusting them by regression within breed group to a common weight and height (Tables 5, 6, 7, and 8). The h^2's for pelvic width were greater than h^2's for pelvic height in both analyses for both sexes (Tables 5, 6, 7 and 8). The rg's between pelvic measures and 368-day weight and 368-day height were greater in both males and females than the rp's among these traits (Tables 5 and 7).

Among females that produced calves as two-yr-olds, in the analysis including all calves, the rg's for pelvic width with 368-day weight, 368-day height, calf birth weight and calving difficulty score were, respectively, .57, .72, .38, and -.42

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(Table 9). The rg's of calf birth weight with 368-day weight and 368-day height were, respectively, .40 and .44 but the rg's of 368-day weight and 368-day height with calving difficulty score approached 0 (Table 9). The rg and rp of calf birth weight with calving difficulty score were, respectively, .50 and .51 (Table 9). The rp's of pelvic measures with both measures of dystocia approached 0 (Table 9). Adjusting pelvic measures and measures of dystocia to a common calf birth weight within sex resulted in little increase in the rp's between pelvic measures and measures of dystocia, whereas, the rg of pelvic width with calving difficulty score was increased from -.42 to -.80 (Tables 9 and 10). The rg's between calf birth weight and calving difficulty score were .17 and .70 for females producing female and male calves, respectively (Tables 11 and 12).

The low rp's between pelvic measures and both measures of dystocia (calving difficulty score and calving difficulty percentage) suggest that selecting replacement females based on their pelvic measures at 320 days would have little effect on dystocia of either their male or female progeny at first parturition. The magnitude of the rg's suggests that optimum weighting of pelvic width at 320-days along with 368-day weight and 368-day height with negative weighting of calving difficulty score and calf birth weight in a selection index should result in response to selection for reduced dystocia while maintaining 368-day weight and 368-day height. However, because most of the selection opportunity in cattle is among males, the critical question that is *not* addressed in this study is the rg between pelvic measures in bulls and first parturition dystocia of their daughters.

Table 1—Number of sires and individuals and least squares breed group means for pelvic measures and size – females

Breed group	Number sires	Number individuals	Pelvic height (cm)	Pelvic width (cm)	Pelvic area (cm)	368-day weight (lb)	368-day height (in)
Overall mean	552	5,715	12.8	11.2	144.2	679	47.2
Red Poll	34	356	12.6	11.0	138.4	633	45.7
Hereford	28	334	11.8	10.3	122.9	593	44.5
Angus	42	400	12.1	10.2	123.7	633	44.9
Limousin	36	350	12.6	10.9	138.4	635	47.2
Braunvieh	39	317	13.3	11.9	158.2	708	48.8
Pinzgauer	31	313	13.2	11.7	155.2	706	48.4
Gelbvieh	39	325	13.0	11.6	152.0	710	48.8
Simmental	37	298	12.8	11.6	149.5	712	49.2
Charolais	37	368	13.4	11.8	157.8	719	48.8
MARC I	84	869	13.1	11.6	153.1	719	48.4
MARC II	79	959	12.6	11.1	141.0	703	47.2
MARC III	66	826	12.6	11.0	140.3	681	46.1
D.05[a]			.21	.20	4.4	17.6	.4

[a] D.05 is the approximate difference between breed group means required for significance.

Table 2—Number of sires and individuals and least squares breed group means for pelvic measures and size - males

Breed group	Number sires	Number individuals	Pelvic height (cm)	Pelvic width (cm)	Pelvic area (cm)	368-day weight (lb)	368-day height (in)
Overall mean	503	4,531	12.4	11.0	136.7	891	49.2
Red Poll	29	207	12.2	10.9	134.4	836	48.0
Hereford	25	243	11.6	10.2	119.4	763	46.1
Angus	39	309	11.8	10.3	122.6	800	46.4
Limousin	32	254	12.6	10.9	137.8	825	49.2
Braunvieh	36	220	12.5	11.4	143.6	930	50.4
Pinzgauer	27	220	13.0	11.5	150.0	937	49.6
Gelbvieh	33	257	12.5	11.3	141.8	955	50.4
Simmental	30	225	12.6	11.3	142.7	948	50.8
Charolais	35	229	12.6	11.4	145.0	942	50.8
MARC I	81	783	12.3	11.0	136.4	917	49.6
MARC II	73	910	12.2	10.8	132.5	926	48.8
MARC III	63	674	12.4	10.8	134.8	902	48.0
D.05[a]			.30	.27	6.33	25.1	.5

[a] D.05 is the approximate difference between breed group means required for significance.

Table 3—Number of sires and individuals and least squares breed group means for calf birth weight and dystocia of females producing calves - sexes combined

Breed group	Number sires	Number individuals	Calf birth weight (lb)	Calving difficulty score[a]	Calving difficulty (%)[b]
Overall mean	438	2,942	84.0	2.9	52.1
Red Poll	29	189	77.8	2.8	58.7
Hereford	20	173	75.2	2.7	48.6
Angus	37	225	71.4	2.3	40.9
Limousin	28	154	78.5	1.9	29.1
Braunvieh	34	182	93.5	3.8	68.9
Pinzgauer	27	179	94.4	3.7	67.9
Gelbvieh	34	193	87.3	3.4	59.9
Simmental	32	165	86.2	2.9	52.0
Charolais	33	177	87.3	2.3	39.0
MARC I	56	424	89.7	3.1	56.7
MARC II	53	405	85.8	3.1	56.3
MARC III	55	476	82.0	2.7	47.3
D.05[c]			3.5	.60	14.5

[a] 1 = no difficulty, 2 = little difficulty by hand, 3 = little difficulty with calf jack, 4 = slight difficulty with calf jack, 5 = moderate difficulty with calf jack, 6 = major difficulty with calf jack, 7 = caesarean birth.

[b] Percent requiring assistance.

[c] D.05 is the approximate difference between breed group means required for significance.

Table 4—Least squares breed group means by sex for calf birth weight and dystocia

Breed group	Calf birth wt (lb)		Calving difficulty score[a]		Calving difficulty (%)[b]	
	Males	Females	Males	Females	Males	Females
Overall mean	87.1	80.9	3.4	2.3	64.6	38.8
Red Poll	80.5	75.2	3.2	2.4	68.9	47.8
Hereford	77.4	73.0	3.4	2.0	68.9	25.1
Angus	73.4	69.2	2.7	2.0	53.3	28.3
Limousin	81.1	75.8	2.2	1.7	37.1	20.7
Braunvieh	98.3	88.2	4.7	2.9	83.0	54.5
Pinzgauer	97.9	90.6	4.3	3.0	79.8	55.5
Gelbvieh	90.6	83.8	4.2	2.6	76.5	42.3
Simmental	89.5	82.7	3.6	2.1	69.2	33.4
Charolais	89.5	85.1	2.6	2.0	45.1	31.8
MARC I	92.6	86.6	3.6	2.6	65.9	47.1
MARC II	88.4	83.3	3.7	2.5	68.0	44.6
MARC III	85.3	78.5	3.3	2.1	60.0	34.4
D.05[c]	4.8	4.6	.9	.8	19.4	21.4

[a, b, c,] See footnotes for Table 5.

Table 5—Estimates of heritability (h^2) of and genetic (rg) and phenotypic (rp) correlations among pelvic measures and size - females [a,b,c]

	Pelvic height (cm)	Pelvic width (cm)	Pelvic area (cm)	368-day weight (lb)	368-day height (in)
Pelvic height (cm)	.14 ± .03	.64 ± .08	.88 ± .03	.46 ± .10	.70 ± .09
Pelvic width (cm)	.59	.25 ± .04	.92 ± .02	.53 ± .08	.60 ± .07
Pelvic area (cm)	.88	.90	.20 ± .04	.54 ± .08	.70 ± .07
368-d weight (lb)	.33	.37	.39	.32 ± .04	.72 ± .04
368-d height (in)	.33	.35	.38	.64	.44 ± .04

[a] Estimates of h^2 on diagonal.

[b] Estimates of rg above diagonal.

[c] Estimates of rp below diagonal.

Table 6—Estimates of heritability (h^2) of and genetic (rg) and phenotypic (rp) correlations among pelvic measures – adjusted to common height and weight - females[a,b,c]

	Pelvic height (cm)	Pelvic width (cm)	Pelvic area (cm)
Pelvic height (cm)	.08 ± .03	.44 ± .14	.79 ± .07
Pelvic width (cm)	.52	.19 ± .03	.90 ± .03
Pelvic area (cm)	.86	.88	.14 ± .03

[a] Estimates of h^2 on diagonal.
[b] Estimates of rg above diagonal.
[c] Estimates of rp below diagonal.

Table 7—Estimates of heritability (h^2) of and genetic (rg) and phenotypic (rp) correlations among pelvic measures and size - males[a,b,c]

	Pelvic height (cm)	Pelvic width (cm)	Pelvic area (cm)	368-d weight (lb)	368-d weight (in)
Pelvic height (cm)	.46 ± .05	.80 ± .03	.93 ± .01	.31 ± .08	.42 ± .07
Pelvic width (cm)	.55	.60 ± .06	.96 ± .01	.32 ± .07	.42 ± .06
Pelvic area (cm)	.91	.84	.62 ± .06	.32 ± .07	.43 ± .06
368-d weight (lb)	.28	.30	.33	.42 ± .05	.62 ± .05
368-d height (in)	.25	.21	.27	.66	.55 ± .05

[a, b, c] See footnotes for Table 6.

Table 8—Estimates of hertitability (h^2) of and genetic (rg) and phenotypic (rp) correlations among pelvic measures – adjusted to common height and weight – males[a,b,c]

	Pelvic height (cm)	Pelvic width (cm)	Pelvic area (cm)
Pelvic height (cm)	.40 ± .05	.78 ± .04	.93 ± .01
Pelvic width (cm)	.63	.52 ± .05	.96 ± .01
Pelvic area (cm)	.90	.90	.54 ± .05

[a, b, c] See footnotes for Table 6.

Table 9—Estimates of heritability (h^2) of and genetic (rg) and phenotypic (rp) correlations among pelvic measures, size calf birth weight and dystocia for females producing calves - all calves[a,b,c]

	Pelvic height (cm)	Pelvic width (cm)	Pelvic area (cm)	368-d weight (lb)	368-d height (in)	birth weight (lb)	Cal Calving difficulty score	Calving difficulty (%)
Pelvic height (cm)	.17 ± .05	.62 ± .11	.86 ± .05	.55 ± .13	.79 ± .13	.70 ± .19	.10 ± .27	.02 ± .32
Pelvic width (cm)	.58	.41 ± .06	.93 ± .02	.57 ± .09	.72 ± .08	.38 ± .13	-.42 ± .21	-.24 ± .25
Pelvic area (cm)	.88	.89	.30 ± .06	.62 ± .09	.81 ± .09	.55 ± .14	-.26 ± .22	-.19 ± .27
368-d weight (lb)	.31	.37	.39	.43 ± .06	.74 ± .06	.40 ± .12	.01 ± .19	.27 ± .24
368-d height (in)	.32	.35	.38	.62	.39 ± .06	.44 ± .12	.03 ± .19	.29 ± .25
Calf birth weight (lb)	.12	.11	.13	.23	.24	.25 ± .06	.50 ± .17	.52 ± .23
Calving difficulty score	-.06	-.11	-.09	.00	-.06	.51	.12 ± .05	.90 ± .09
Calving difficulty (%)	-.03	-.08	-.07	.01	-.03	.40	.85	.07 ± .05

[a, b, c] See footnotes for Table 6.

Table 10—Estimates of heritability (h^2) of and genetic (rg) and phenotypic (rp) correlations among pelvic measures, size, calf birth weight and dystocia for females producing calves - adjusted to common calf birth weight - all calves[a,b,c]

	Pelvic height (cm)	Pelvic width (cm)	Pelvic area (cm)	368-d weight (lb)	368-d height (in)	Calving difficulty score	Calving difficulty (%)
Pelvic height (cm)	.14 ± .05	.59 ± .12	.84 ± .06	.47 ± .15	.74 ± .15	-.47 ± .32	-.58 ± .44
Pelvic width (cm)	.57	.39 ± .06	.93 ± .02	.53 ± .09	.69 ± .09	-.80 ± .26	-.58 ± .33
Pelvic area (cm)	.87	.89	.27 ± .06	.56 ± .10	.17 ± .10	-.77 ± .28	-.68 ± .38
368-d weight (lb)	.29	.36	.37	.41 ± .06	.70 ± .07	-.29 ± .20	.01 ± .24
368-d height (in)	.30	.33	.36	.60	.36 ± .06	-.30 ± .14	.00 ± .25
Calving difficulty score	-.14	-.19	-.19	-.14	-.14	.12 ± .05	.90 ± .11
Calving difficulty (%)	-.09	-.14	-.13	-.09	-.22	.82	.07 ± .05

[a] Estimates of h^2 on diagonal.
[b] Estimates of rg above diagonal.
[c] Estimates of rp below diagonal.

Table 11—Estimates of heritability (h^2) of and genetic (rg) and phenotypic (rp) correlations among pelvic measures, size, calf birth weight and dystocia - female calves[a,b,c]

	Pelvic height (cm)	Pelvic width (cm)	Pelvic area (cm)	368-d weight (lb)	368-d height (in)	Calf birth weight (lb)	Calving difficulty score	Calving difficulty (%)
Pelvic height (cm)	.25 ± .10	.54 ± .17	.84 ± .08	.48 ± .23	.56 ± .22	.59 ± .30	-.03 ± .40	.00 ± .42
Pelvic width (cm)	.59	.40 ± .11	.91 ± .04	.60 ± .17	.60 ± .18	.52 ± .25	-.30 ± .36	-.31 ± .38
Pelvic area (cm)	.88	.90	.30 ± .11	.64 ± .19	.68 ± .19	.67 ± .28	-.20 ± .39	-.21 ± .41
368-d weight (lb)	.30	.34	.36	.36 ± .11	.43 ± .18	.40 ± .24	.12 ± .34	.25 ± .37
368-d height (in)	.34	.33	.38	.60	.33 ± .11	.31 ± .25	-.10 ± .36	-.08 ± .37
Calf birth weight (lb)	.15	.10	.14	.20	.23	.25 ± .10	.17 ± .38	.15 ± .41
Calving difficulty score	-.04	-.08	-.07	.00	-.04	.46	.14 ± .10	1.02 ± .08
Calving difficulty (%)	-.03	-.08	-.06	.01	-.01	.40	.90	.13 ± .10

[a] Estimates of h^2 on diagonal.
[b] Estimates of rg above diagonal.
[c] Estimates of rp below diagonal.

Table 12—Estimates of heritability (h^2) of and genetic (rg) and phenotypic (rp) correlations among pelvic measures, size, calf birth weight and dystocia - male calves[a,b,c]

	Pelvic height (cm	Pelvic width (cm)	Pelvic area (cm)	368-d weight (lb)	368-d height (in)	Calf birth weight (lb)	Calving difficulty score	Calving difficulty (%)
Pelvic height (cm)	.17 ± .05	.62 ± .11	.86 ± .05	.55 ± .13	.79 ± .13	.70 ± .19	.10 ± .27	.02 ± .32
Pelvic height (cm)	.17 ± .09	.46 ± .19	.80 ± .09	.65 ± .22	.82 ± .23	.60 ± .33	.21 ± .37	.20 ± .51
Pelvic width (cm)	.57	.44 ± .10	.91 ± .04	.58 ± .13	.73 ± .13	.41 ± .21	-.18 ± .25	-.04 ± .33
Pelvic area (cm)	.87	.89	.33 ± .10	.67 ± .14	.84 ± .14	.53 ± .23	-.07 ± .28	.02 ± .37
368-d weight (lb)	.33	.39	.41	.42 ± .10	.88 ± .08	.44 ± .19	-.11 ± .24	.20 ± .34
368-d height (in)	.31	.36	.38	.63	.45 ± .10	.56 ± .19	-.20 ± .25	.20 ± .34
Calf birth weight (lb)	.11	.12	.13	.27	.25	.26 ± .10	.70 ± .19	.71 ± .33
Calving difficulty score	-.08	-.13	-.12	.01	-.07	.54	.20 ± .09	.83 ± .15
Calving difficulty (%)	-.04	-.08	-.07	.02	-.04	.42	.84	.10 ± .09

[a] Estimates of h^2 on diagonal.
[b] Estimates of rg above diagonal.
[c] Estimates of rp below diagonal.

Twinning in Cattle

Keith E. Gregory, Sherrill E. Echternkamp, L. Dale Van Vleck, and Larry V. Cundiff[1]

Introduction

Rate of reproduction has a major impact on life cycle costs of production of different animal species and upon their competitiveness for different types of production resources. For example, the average beef cow is capable of producing about .7 of her body weight per year in progeny market weight; comparable values are about 8 in pigs, more than 70 in meat chickens and more than 1,000 in catfish. More than 50% of the feed nutrients used by the beef cattle industry of the United States are needed to meet maintenance requirements of reproducing females. The comparable value in meat chickens is less than 3%. Further, high producing dairy cows produce five times as much milk protein per unit of feed as beef cattle produce as beef protein. Small differences in reproduction rate of beef cattle can have a major effect on costs of production and on the production resources for which beef cattle are competitive. Results from experimentation and computer simulation suggest that input costs per unit of beef output could be reduced by from 20 to 30% for the proportion of the herd that produces twins.

A twinning technology would require a highly intensive production system. Initial calculations indicate that a twinning rate of about 40% may be needed to make a twinning technology economically viable. Because of the time and effort required to develop a population with a twinning frequency of 40% or greater, private sector interests are not likely to make the investment required. Thus, the development of a population of cattle that has a high breeding value for twinning (i.e., 40% or greater) and is competitive in production and carcass traits is an essential component of a twinning technology for use by the beef cattle industry.

Procedure

The effort was implemented as a formal project in 1981. There were 307 foundation females in the project with 96 originating in private sector herds and 211 originating from other projects at the Research Center. Twinning records of foundation cows before and after entry into the twinning project are shown in Table 1. Foundation sires have included sons of the foundation cows, and a Charolais sire and a Pinzgauer sire whose daughters produced twins at a high frequency in another project at the Research Center. Also, semen from Swedish Friesian (8 sires), Swedish Red and White (5 sires), and Norwegian Red (2 sires) whose daughters had produced twins at a frequency of from 8-13% has been imported and used in the project.

Breeds represented in the project include: (1) Holstein, (2) Simmental, (3) Charolais, (4) Brown Swiss, (5) Braunvieh, (6) Pinzgauer, (7) Gelbvieh, (8) Swedish Friesian, (9) Swedish Red and White, (10) Norwegian Red, (11) Shorthorn, (12) Hereford, and (13) Angus. The intent was to sample breeds that twin at a relatively high frequency. The Shorthorn, Hereford and Angus do not meet this criterion and were introduced as residual from grade-up programs to breeds that were introduced into the project and produce twins at a relatively high frequency.

A multiple trait (ovulation rate and twinning rate) repeated records animal model was used to predict breeding value (PBV) for twinning rate. This procedure combines information on individuals and relatives for ovulation and twinning rate and was implemented with selections and matings made in 1990.

About 750 calving cows are included in the project. Calving is both spring and fall. Mating seasons are 70 days. About one-fourth of females (heifers and cows) with highest predicted breeding value for twinning (PBV) are mated by artificial insemination (AI) to progeny proven sires for the full breeding season. These matings provide the young sires to be progeny tested. The remaining heifers are mated by natural service to young high PBV but unproved sires for the full mating season and remaining cows are mated AI to young high PBV but unproved sires for 40-42 days and cleaned up by natural service mating to young high PBV but unproved sires for 28-30 days.

Breeding assignments to young high PBV but unproved sires are made with the intent of obtaining 8-10 daughter and 8-10 son progeny conceived in both spring and fall mating seasons.

All females are examined by ultrasonography 40-80 days postconception to determine number of fetuses.

Calves are weaned at an average age of 140-150 days (late August for spring born calves and late January for fall born calves). Calves are creep fed and both sexes are fed a growing diet from weaning to an average age of 200 days.

At an average age of 200 days candidate males (about 50 per year with highest PBV) are identified and fed for 140 days on a diet of 2.69 Mcal ME/kg of dry matter and 12.88 C.P. at which time final decisions are made on bulls (about 25 per year) to be retained for progeny testing. Males not identified at an average age of 200 days to be developed out as potential sires for progeny testing are castrated and grouped by weight into three weight classes (pens) and fed a diet of high energy density (3.12 Mcal ME/kg of dry matter) from an average pen weight of 600-625 lb to a final pen weight of 1300-1325 lb. Growth and carcass data are obtained on all castrate males.

Heifers are developed on a standard breeding heifer development program. Starting at an average age of about 12 months, ovulation rate is determined by rectal palpation of corpora lutea for 8-10 estrous cycles. Heifers are mated first at an average age of about 1.6 yr. Fall born heifers are mated in spring and spring born heifers are mated in fall to produce their first calves at an average age of 2.5 yr.

Between 150 and 260 units of semen are collected and frozen on each young high PBV bull included in a progeny test. About 25 young sires are progeny tested each year. Young sires are used in progeny test matings at 1.0, 1.5, and 2.0 yr so that progeny test information on ovulation rate of daughters and growth and carcass traits of sons is available when bulls are not older than 4.5 years.

In order to control rate of inbreeding, an average of five males from the 25 progeny tested each year are mated subsequently by AI in "elite" matings to approximately 25% of females with highest PBV for twinning. This mating procedure will result in use of about 25 sires in "elite" matings in each generation. Rate of increase in inbreeding should not be greater than about .5% per generation or about 5% in 10 generations, i.e., 50 years. Also, matings of progeny

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proven sires to "elite" (high PBV) females are made so that the contribution of a single breed will not be greater than 25-30% in any individual over the longer term. This mating plan should result in the retention of high levels of heterosis for bioeconomic traits.

Results

Twinning rate of daughters of foundation cows averaged 11% (Table 2). Estimates of heritability of twinning rate based on use of three methods ranged from 6-9%. Analysis of records provided by the Swedish Government Artificial Insemination Organization indicated that twinning in cattle is inherited as a quantitative trait, i.e., a relatively large number of genes are likely involved, each with relatively small effects.

At the time the project was implemented it was recognized that a predictor of genetic capacity to produce twins that could be evaluated at a young age was needed in order to make significant progress in increasing twinning rate. Such a predictor is needed to reduce generation interval and because it is not possible to keep all females to evaluate their twinning rate on more than a limited number of parturitions. The most obvious predictor to investigate was ovulation rate in puberal heifers because it was believed that ovulation rate was likely the most limiting factor.

The following factors confirmed the potential value of using ovulation rate in puberal heifers as an indirect selection criterion for twinning rate.

1. As validated by laparoscopy, ovulation rate can be determined with high precision (greater than 85%) by rectal palpation of corpora lutea.
2. The effects of ovulation rate (1 or 2) on embryonic and fetal survival are small and nonsignificant.
3. Unilateral and bilateral multiple ovulations do not differ in embryonic and fetal survival.
4. Heritability of ovulation rates in individual estrous cycles of puberal heifers averaged .16 in one study. Heritability of ovulation rate was .07 for a single estrous cycle and .34 for an average of 7.9 estrous cycles in another study.
5. Estimates of genetic correlation between averages for ovulation rate in five or more estrous cycles in puberal heifers and subsequent twinning rate approached unity.
6. Thus, by observing ovulation rate in puberal heifers for ~8 estrous cycles we know as much about their genetic value for twinning as we would know after a lifetime of twinning rate observations.

Constraints to a Twinning Technology. The following constraints must be at least partially alleviated in order for a twinning technology to be economically viable.

1. Greater dystocia – 35% vs 23%.
2. More retained placentas – 21.5% vs 2.8%.
3. Longer postpartum interval – 77 vs 63 days.
4. Lower subsequent conception rates – 71% vs 85%.
5. More days to pregnancy – 93 vs 85 days.
6. Reduced perinatal survival – 82 vs 97%.

Some Considerations in Alleviating Constraints. Days to estrus, conception rate, and days to pregnancy were *not* affected by number of calves reared (1 *vs* 2) in cows birthing twins. These findings were interpreted to result from inadequate prepartum nutrition in cows birthing twins. Results from an experiment conducted at the University of California-Davis showed that cows gestating twins did not have sufficient body capacity to consume enough feed of a low energy density diet to meet their nutritive requirements in the last trimester of gestation. Survival at birth was greater for single than for twin born calves (97% vs 82%) but twins and singles did not differ in postnatal survival. When dystocia was experienced, survival to birth was 95% for singles but only 73% for twins. When dystocia was *not* experienced, survival to birth was 99% for singles and 92% for twins. The greater difference in survival between twins and singles when dystocia was experienced than when dystocia was *not* experienced is believed to result largely from failure to provide timely assistance at parturition.

Thus, the identification of cows gestating twins to provide for their: (1) higher nutritive requirements in the last trimester of gestation, and (2) higher calving assistance requirement at parturition is needed to make twinning an economically viable production technology. Ultrasonography at 40-80 days post-conception can give high precision in identifying cows gestating twins.

A summary of the effects of age (months) on ovulation rate of puberal heifers is presented in Table 3. These results show a major effect of age to 11 months, some effect to 12 months but no effect of age between 12 and 18 months.

A summary of change in ovulation rate in puberal heifers by year of birth is presented in Table 4 for each birth year since 1984 when ovulation rate determinations were started. Large changes in ovulation rate were observed to birth year 1987 but little change was observed for birth years subsequent to 1987.

A summary of the effects of month on ovulation rate of puberal heifers is presented in Table 5. Ovulation rates were highest in June and through fall and early winter months of September through December. The months with highest ovulation rates coincide, generally, with spring (June and July) and fall (November and December) breeding seasons.

A summary of twinning rate by *year of birth* since 1981 of females born in the project is presented in Table 6. A reasonably consistent rate of increase is shown through birth year 1986. Females born in 1987, 1988 and 1989 show a much higher twinning rate than females born in prior years. Our interpretation is that this increased twinning rate is likely accounted for by the greater use of high PBV progeny proven sires and the result of consideration of ovulation rate in puberal heifers in selection decisions.

A summary of twinning rate of females born in the project by *year of calving* is presented in Table 7. A reasonably consistent rate of increase is shown through 1989 calving year. Females calving in 1990 and 1991 showed a marked increase in twinning rate relative to prior years. Again, this likely reflects the greater use of high PBV progeny proven sires and the consideration of ovulation rate in puberal heifers.

A summary of twinning rate by age of female for 1991 calving (spring and fall) is shown in Table 8. Twinning rate normally increases in second and third parturitions. For 1991 and 1992, age of dam adjustments for twinning rate to a mature basis were: 2 yr olds = 16.3% and 3 yr olds = 2.3% after adjusting records to a common predicted breeding value. The lower twinning rate of females six or more yr old indicates that the younger females had a higher genetic capacity to produce twins and suggests a high rate of genetic improvement in twinning rate.

Providing the germplasm (breeding stock) to the beef cattle industry is required for the implementation of a twinning technology. Thus, young males (two yr old) that are in the process of being progeny tested are sold at public auction with a repurchase option that will be exercised within three years for highly superior males. In addition to PBV for twin-

ning rate, growth rate of all progeny and carcass traits of castrate male progeny will be considered. Males on which the repurchase option is exercised will be licensed to an artificial insemination organization that will offer semen to the beef cattle industry.

For greater detail see:

1. Keith E. Gregory, S. E. Echternkamp, G. E. Dickerson, L. V. Cundiff, R. M. Koch, and L. D. Van Vleck. 1990. Twinning in cattle: I. Foundation animals and genetic and environmental effects on twinning rate. J. Anim. Sci. 68:1867-1876.
2. Sherrill E. Echternkamp, K. E. Gregory, G. E. Dickerson, L. V. Cundiff, R. M. Koch, and L. D. Van Vleck. 1990. Twinning in cattle: II. Genetic and environmental effects on ovulation rate in puberal heifers and postpartum cows and the effects of ovulation rate on embryonic survival. J. Anim. Sci. 68:1877-1888.
3. Keith E. Gregory, S. E. Echternkamp, G. E. Dickerson, L. V. Cundiff, R. M. Koch and L. D. Van Vleck. 1990. Twinning in Cattle: III. Effects of twinning on dystocia, reproductive traits, calf survival, calf growth and cow productivity. J. Anim. Sci. 68:3133-3144.
4. L. Dale Van Vleck, K. E. Gregory and S. E. Echternkamp. 1991. Ovulation rate and twinning rate in cattle: Heritabilities and genetic correlation. J. Anim. Sci. 69:3213-3219.
5. L. Dale Van Vleck, K. E. Gregory and S. E. Echternkamp. 1991. Prediction of breeding values for twinning rate and ovulation rate with a multiple trait, repeated records animal model. J. Anim. Sci. 69:3959-3966.

Table 1—Foundation cows in twinning project

	Records before entry	Records after entry
Number	307	307
Progeny	1,348	1,055
Parturitions/cow	3.0	2.9
Progeny/parturition	1.50	1.17

Table 2—Twinning rate of daughters of foundation cows

	All Data
No. daughters	710
No. parturitions	1,374
Parturitions/daughter	1.94
Progeny/parturition	1.11

Table 3—Summary of ovulation rate of puberal heifers by age class

Age months	Mean ovulation rate
≤ 11	1.06
12	1.12
14	1.16
16	1.16
≥ 18	1.17

Table 4—Ovulation rate of puberal heifers by year of birth

Year of birth	Mean ovulation rate
1984	1.09
1985	1.10
1986	1.11
1987	1.15
1988	1.16
1989	1.16
1990	1.14

Table 5—Summary of ovulation rate of puberal heifers by month

Month	Mean ovulation rate
January	1.14
February	1.13
March	1.11
April	1.14
May	1.12
June	1.16
July	1.14
August	1.13
September	1.16
October	1.16
November	1.16
December	1.15

Table 6—Summary of twinning rate by year of birth (Females born in project)

Birth year	No. parturitions	No. born/ parturition
Overall mean	3,618	1.18
1981	321	1.08
1982	283	1.11
1983	507	1.10
1984	628	1.12
1985	705	1.15
1986	456	1.17
1987	394	1.29
1988	234	1.35
1989	90	1.24

Table 7—Summary of twinning rate by year of calving (Females born in project)

Calving year	No. parturitions	No. born/ parturition
Overall mean	3,618	1.10
1981	47	1.00
1982	41	1.06
1983	61	1.10
1984	109	1.06
1985	215	1.06
1986	292	1.08
1987	446	1.09
1988	435	1.11
1989	555	1.14
1990	654	1.19
1991	763	1.24

Table 8—Summary of twinning rate for 1991 calving by age class (Females born in project)

Age	No.	No. born/ parturition
Overall Mean	763	1.25
2	192	1.21
3	141	1.30
4	111	1.30
5	117	1.25
≥ 6	202	1.16

Mortality and Cold Tolerance of Calves with Different Ratios of *Bos indicus* to *Bos taurus* Inheritance

Maurie J. Josey, Larry V. Cundiff, Robert M. Koch, Keith. E. Gregory, and G. LeRoy Hahn[1]

Introduction

Results from the Germplasm Evaluation Program (GPE) at the Roman L. Hruska U. S. Meat Animal Research Center (MARC) have shown that significant differences exist among cows representing breeds of diverse biological types in such production traits as 200-day weaning wt per cow in the breeding herd. Production of *Bos indicus* F_1 cross cows (Brahman and Sahiwal sired crosses out of Hereford and Angus dams) was equalled only by F_1 cross cows sired by large size dual purpose breeds (Brown Swiss, Gelbvieh, Simmental, Holstein and Maine Anjou crosses out of Hereford and Angus dams) excelling in milk production and genetic potential for growth. Indications are that the advantages of *Bos indicus* crosses are associated with greater heterosis found in *Bos indicus* x *Bos taurus* crosses than is found in crosses of two *Bos taurus* breeds. In the GPE program, F_1 cross cows by diverse sire breeds were compared when they were raising terminal three-breed cross calves by bulls of an unrelated *Bos taurus* breed (e.g., Simmental or Brown Swiss). Thus, progeny of *Bos indicus* sired F_1 cross cows were 25% *Bos indicus* and 75% *Bos taurus* compared to progeny of 100% *Bos taurus* inheritance for all other F_1 cross cows. Matings were made to determine the optimum proportion of *Bos indicus* inheritance in a temperate environment. Results for mortality and cold tolerance of calves containing increasing proportions of *Bos indicus* (O, 25, 50 and 75% Brahman or Sahiwal) relative to *Bos taurus* (100, 75, 50 and 25% Angus, Hereford or Pinzgauer) inheritance are presented in this report.

Procedure

Data were obtained on 1,010 calves born at MARC in the spring (late February to early May) of 1983, 1984, 1985 and 1986. Reciprocal backcross and F_2 matings as outlined in Table 1 provided calves with 0:100, 25:75, 50:50 and 75:25 ratios of *Bos indicus* to *Bos taurus* inheritance. The purebred A, H, P, B and S sires used to produce the F_1 cross dams (Cycle III, Phase 2) were also used to produce the backcross and F_2 cross calves (Cycle III, Phase 4) evaluated in this study. Sire to daughter matings were avoided to prevent inbreeding. The F_1 (HA, AH, PH, PA, BH, BA, SH, and SA) sires used to produce F_2 calves were produced in 1980 by mating the original purebred A, H, P, B and S sires to Hereford and Angus dams from the base herd of the Germplasm

Evaluation Program

Cows were maintained on improved pastures and fed hay in winter. All cows were observed for calving difficulty and assistance was given when necessary. All calves were tattooed and tagged for identification and male calves were castrated within 24 hr of birth. When signs of cold stress such as increased shivering, glazed eyes, huddling and decreased activity were observed, calves were placed in a heat chamber for 30 min to 3 hr. The chamber was rectangular in shape, enclosed on three sides, open in the front and elevated about 30 inches above the ground. Hot air was forced upward into the chamber through an expanded metal floor by a forced air propane space heater placed in front of the chamber at a distance manually regulated to maintain ambient temperature within the chamber at about 100-120 degrees Fahrenheit.

Data were recorded on calf mortality between birth and weaning (1 = died, 0 = alive), heat chamber usage (1 = used, 0 = not used), and the combination of calf mortality or heat chamber usage (1 = calf died or survived only with aid of heat chamber, 0 = calf lived without aid of heat chamber). The rationale for the latter trait assumes calves would have died as a result of cold stress had they not been revived in the heat chamber.

Weather records obtained by the University of Nebraska South Central Station at MARC were used to study effects of avg daily temperature (classified into five levels: <30, 30 to <36, 36 to <41, 41 to <46, 46 to <52 and ≥ 52°F) and precipitation (classified into two levels: ≤ .1 in versus > .1 in water equivalent) on the day of the calf's birth on mortality and on heat chamber usage.

Ko was calculated according to the formula developed by Sipple and Passel (1945, Proc. Amer. Phil. Soc. 89: 177-199),

$$Ko = (10v^{1/2} - v + 10.45)(33 - Td),$$

where Ko = heat loss in $Kcal/m^2/hr$, v = mean daily wind speed (m/s) and Td = avg daily temperature (°C).

Data were analyzed by least squares procedures with a model (model 1) that included fixed effects for *Bos indicus*:*Bos taurus* ratio, avg daily temperature class, precipitation class, and their two- and three-way interactions. Model 2 was the same except that a fixed effect for a wind chill factor (Ko classified into five levels: <700, 700 to 799, 800 to 899, 900 to 999, >999) on the day of each calf's birth was substituted for temperature class.

Results

Table 2 presents means for breed group (B), avg daily temperature classes and breed group-temperature subclasses. Mortality increased significantly as the proportion of *Bos indicus* and avg temperature on the day of birth decreased, especially in calves that were 50% or more *Bos indicus*. Mortality for *Bos taurus* calves (0:100) was not significantly different from that for 25% *Bos indicus* (25:75) calves, even on the coldest days. Heat chamber usage and the combination of mortality or heat chamber usage also increased significantly in all breed groups as avg temperature on the day of birth decreased, but especially as proportion of *Bos indicus* inheritance increased.

Precipitation on the day of birth did not significantly affect mortality (Table 3). On the other hand, heat chamber usage and the combination of mortality or heat chamber usage were significantly affected by precipitation (Table 3). These results indicate that precipitation effects would have increased calf mortality, especially in calves with 50% or more *Bos indicus* inheritance, if the calves had not been placed in a heat chamber.

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A significant interaction was noted among breed group-temperature-precipitation classes. As proportion of *Bos indicus* inheritance increased, the trend for mortality, heat chamber usage, and their combination to increase with declining temperatures was amplified in the presence of precipitation. Mortality, heat chamber usage and their combination were very high on cold-wet days.

Results for windchill (Ko) classes were very similar to those for temperature classes (Table 4). Windchill accounted for slightly more, but not significantly more, variation in mortality, heat chamber usage and the combination of mortality or heat chamber usage than temperature classes (Table 2). Mortality increased significantly as the proportion of *Bos indicus* and windchill on the day of a calf's birth increased.

Conclusions.

Results of this study reveal that calves with 50% or more *Bos indicus* inheritance are not as well adapted as calves with only 25% or less *Bos indicus* inheritance to calving conditions which can be characterized as cold (mean daily temperature 41°F or less), cold and wet (46°F or less combined with .1 inch or more precipitation), or cold and windy (Ko > 800 Kcal/m^2/hr). Results for heat chamber usage and for the combination of mortality or heat chamber usage indicate that losses would have been especially severe without use of the heat chamber. Fortunately, a majority of calves are born under more favorable calving conditions than these, even in March and April in Nebraska. However, to avoid calf losses associated with cold stress, the calving season should be changed to coincide with warmer months of the year (May to October at MARC) or matings should be made to restrict the level of *Bos indicus* inheritance to 25% or less.

Table 1—Mating plan to produce calves in 1983-1986 calf crops and their *Bos indicus*:*Bos taurus* inheritance (I:T)[a]

Breed of sire	Breed of dam								I:T
	AH	HA	PH	PA	BH	BA	SH	SA	
A	X	X		X					0:100
H	X	X	X						0:100
HA&AH	X	X							0:100
P			X	X					0:100
PH			X						0:100
PA				X					0:100
A						X		X	25:75
H					X		X		25:75
BH					X				50:50
BA						X			50:50
SH							X		50:50
SA								X	50:50
B					X	X			75:25
S							X	X	75:25

[a] Matings among *Bos indicus* (Brahman, B; Sahiwal, S) and *Bos taurus* (Hereford, H; Angus, A; Pinzgauer, P) breeds provided calves for this study. Two letters denote sire and dam breed of F_1 crosses.

Table 2—Means for breed group and average daily temperature classes

Temp. class (F°)	*Bos indicus*:*Bos taurus* ratio				Mean
	0:100	25:75	50:50	75:25	
	Number of calves				
52 or more	118	33	57	42	250
46 to <52	71	26	42	17	156
41 to <46	75	11	28	26	140
36 to <41	73	29	44	25	171
30 to <36	89	26	50	21	186
less than 30	53	14	28	12	107
Total	479	139	249	143	1010
	Mortality, birth to weaning, %				
more than 52	2	0	4	1	2
46 to <52	2	5	0	4	2
41 to <46	5	0	4	36	11
36 to <41	2	4	4	41	13
30 to <36	1	2	22	37	16
less than 30	3	8	12	9	8
Mean	3	3	8	21	
	Heat chamber required, %				
more than 52	0	0	0	0	0
46 to <52	0	0	10	17	7
41 to <46	4	0	2	27	8
36 to <41	14	17	30	32	23
30 to <36	16	34	54	27	33
less than 30	29	17	66	86	50
Mean	11	11	27	31	
	Mortality or heat chamber, %				
more than 52	2	0	4	1	2
46 to <52	2	5	10	20	9
41 to <46	9	0	6	54	17
36 to <41	16	21	34	52	30
30 to <36	17	36	64	60	44
less than 30	32	25	76	86	55
Mean	13	14	32	46	

Table 3—Means for breed group and precipitation classes

Precipitation class (inches)	*Bos indicus*:*Bos taurus* ratio 0:100	25:75	50:50	75:25	Mean
			Number of calves		
Less than .1	403	122	212	114	851
More than .1	76	17	37	29	159
Total	479	139	249	143	1010
			Mortality, birth to weaning, %		
Less than .1	4	6	12	15	9
More than .1	1	0	3	28	8
Mean	3	3	8	21	
			Heat chamber required, %		
Less than .1	6	10	14	24	14
More than .1	15	13	40	39	27
Mean	11	11	27	31	
			Mortality or heat chamber, %		
Less than .1	10	16	25	33	21
More than .1	16	13	40	58	32
Mean	13	14	32	46	

Table 4—Means for breed group and windchill (Ko) classes

Windchill class (Ko)	*Bos indicus*:*Bos taurus* ratio 0:100	25:75	50:50	75:25	Mean
			Number of calves		
700 or less	228	65	110	75	478
700 to 799	79	26	44	24	173
800 to 899	74	18	45	21	158
900 to 999	57	17	25	16	115
999 or more	41	13	25	7	86
Total	479	139	249	143	1010
			Mortality, birth to weaning, %		
700 or less	2	2	3	10	4
700 to 799	4	4	2	28	10
800 to 899	1	3	20	39	16
900 to 999	1	0	11	54	16
999 or more	4	9	11	0	6
Mean	3	4	9	26	
			Heat chamber required, %		
700 or less	0	0	3	7	3
700 to 799	8	2	5	25	10
800 to 899	3	25	40	29	24
900 to 999	29	45	38	54	41
999 or more	29	14	73	84	50
Mean	14	17	32	40	
			Mortality or heat chamber, %		
700 or less	3	2	6	16	7
700 to 799	12	6	7	53	19
800 to 899	4	28	54	55	35
900 to 999	29	45	43	78	47
999 or more	33	23	82	83	55
Mean	16	21	38	56	

Biological Efficiency Differences Among *Bos taurus* x *Bos taurus* and *Bos indicus* x *Bos taurus* F_1-Cross Cows

Ronnie D. Green, Larry V. Cundiff, Gordon E. Dickerson, and Thomas G. Jenkins[1]

Introduction

Matching germplasm to resources through designed crossbreeding programs can contribute to optimum beef production efficiency. This is particularly true in light of the wide diversity of environmental conditions encountered by beef producers in the U.S. This approach requires considerable knowledge about genetic diversity among breeds in components of performance and furthermore how those components interact to influence life-cycle efficiency in the production setting. It was largely this identified need, coupled with the importation of a number of new breeds from continental Europe, that gave impetus for the establishment of the Germplasm Evaluation (GPE) Program. In Cycles I and II of the GPE program, increases in cow output associated with higher breed potential for growth rate and milk production were largely offset by equivalent or greater increases in feed requirements for maintenance and lactation. Additionally, in Cycle III, output of calf weaned per cow in the breeding herd was high for *Bos indicus* x *Bos taurus* crosses relative to *Bos taurus* crosses. More information is needed to evaluate F_1 cross of *Bos taurus* versus *Bos indicus* x *Bos taurus* sources of germplasm. Therefore, this study was conducted to: 1) estimate input/output components, and 2) estimate life-cycle efficiency of Cycle III breeds representing these types of F_1 cross females.

Procedures

This study was conducted in two phases. The first phase was experimental while the second was modeling in nature. Procedures used are given, in brief, for each phase separately. Both phases involved the study of breed groups of cows resulting from the mating of Hereford (H) or Angus (A) cows to Brahman (Bm-X), H or A (HA-X), Pinzgauer (Pz-X) or Sahiwal (Sw-X) sires.

Phase 1. This phase of the study was designed to estimate all inputs and outputs of F1-cross females during an 18 wk drylotted period while the females were open and nursing calves. Calves were sired by Charolais sires and were born during late March to early May of 1987. Cows and calves were moved to the feedlot at approximately 40 days after calving and were assigned to replicated pens (3 pens/breed group) with 12 pairs per pen (breed of dam of cow, sex of calf and age of calf were balanced within pen). Most cows were 11 or 12 yr of age with a few seven-yr olds included in the Pz-X group. Cows were fed to maintain their initial wt for 126 days commencing at approximately 50 days postpartum. Cow and calf wt were recorded biweekly. If avg pen wt was reduced below the initial wt, feed was increased or visa versa. The avg biweekly daily metabolizable energy (ME) consumption for each pen of cows was adjusted statistically to zero biweekly wt change using regression procedures (based on linear regression of mean daily wt change on mean daily ME consumption for 10 biweekly periods in each pen). The cow diet included corn silage (77%), ground brome hay (19%) and a supplement (4%) which included soybean meal, limestone, dicalcium phosphate, vitamins, aureomycin and trace mineral premix. Calves were allowed access to creep feed (whole oats) but not to dam feed. Creep feed consumption was recorded on biweekly intervals. Milk production was estimated by weigh-suckle-weigh procedures at four times during the study (corresponding to 58, 85, 125 and 160 days of lactation) as well as on a group of 44 cow-calf pairs representing the same breed-cross groups in 1986. Lactation curves and curve parameters were used to estimate daily and total milk yield. Fat thickness and visual condition scores were also assessed at the beginning and end of the experimental period to monitor changes in body composition.

Phase II. The second phase of the study involved development of a deterministic computer model to allow estimation of life-cycle efficiency of these breed groups when used in a totally contained breeding system. The system modeled was one of F_1 cross cows bred to Red Poll sires for their first calving as two-yr olds and Simmental sires thereafter. Replacement females were produced in the system. Input and output avg for each subclass of a herd of females made up of the Cycle III breed groups were utilized from the Phase I results, or were obtained from a pooled analysis of data from females produced in Cycles I-III. Energy inputs for maintenance, lactation, pregnancy and female growth were modeled based on Phase I results and avg literature estimates. Herd age distributions were derived from GPE data to 11 yr of age. Weather and environmental conditions were included as model parameters. Final model computations resulted in total herd life-cycle efficiency calculated as:

$$\text{TVALEFF} = \frac{\text{(cow + calf input, Mcal)}}{\text{(weaned calf + .55 x cull cow output, lb)}}$$

Results

Results from the Phase I experiment are presented in Table 1. Overall avg are given for calf gain, creep feed energy consumption, cow wt, fat thickness, daily milk yield and total energy consumption and overall biological efficiency during the 126-day period. Performance for each breed-cross group is presented as a ratio relative to the overall mean (i.e., a ratio of 112 for progeny energy consumption from HA-X dams indicates that calves in that group consumed 12% more creep ME than the overall avg of 592.2 Mcal ME during the 126 days).

Calf gain relative to cow wt was significantly higher for Bm-X and Sw-X groups than for Pz-X and HA-X groups. Calf creep consumption was inversely related to levels of milk production with the exception of the Pz-X group. This appeared to be due to a lower level of persistency of lactation of this breed group resulting in calves consuming larger amounts of creep feed than other groups late in the study.

Significant differences ($P < .01$) existed between F_1-cross groups in cow size, milk yield and level of fatness. Bm-X cows were the largest and gave the highest daily milk yield. Sw-X females were similar to Bm-X in milk yield and level of fatness but were significantly smaller in mature size. Pz-X cows were intermediate in milk yield and mature size and were similar to those from the HA-X group in level of fatness. HA-X cows were the smallest in size of the four groups and gave the lowest amount of milk per day.

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When inputs and outputs were combined into an estimate of biological energy efficiency (lb calf/gain/total ME intake of cow and calf) for this portion of the annual production cycle, Sw-X and Bm-X breed-cross groups were approximately10% more efficient. This was a function of several factors including lower energy requirements per unit of mature size for the cows and lower creep feed energy consumption with higher wt gains of progeny. In the case of the Sw-X group, breed size complementarity from mating of Charolais sires to smaller mature size females also may have given this group an advantage.

These results are somewhat in contrast to those found in similar work from Cycle II of the GPE program. In that study, efficiency results generally favored smaller mature size females due to increases in calf gain from larger mature size and higher milk levels being offset by increased energy consumption required to support that level of performance. The different results of this study are most likely due to 2-3 times higher levels of heterosis generally observed in *Bos indicus* x *Bos taurus* crosses than in *Bos taurus* crosses. This would have given a distinct advantage in components of efficiency to the Sw-X and Bm-X groups. Additionally, any heterosis advantage from increased longevity would have favored the two *Bos indicus-X* groups since these females were 11-12 yr old during the study (as compared to 7-8 in Cycle II).

When results from Phase I and other results from previous components of the GPE program were combined to model life-cycle efficiency of these breed groups, ranking for TVALEFF (Mcal/lb equivalent) was the same as observed in Phase I but differences between the breed groups were narrowed (20.9, 21.6, 22.5, 22.6 Mcal/kg equivalent for Sw-X, Bm-X, Pz-X and HA-X groups, respectively. There still appeared to be an approximate 6% advantage in efficiency for the two *Bos indicus-X* groups over this *Bos taurus-X* counterparts after accounting for all inputs and outputs of the system. This is practically significant given that this system was modeled under conditions (south central Nebraska) to which these breed groups are not naturally adapted and indicates the seeming importance of advantages offered from additional heterosis gained in *Bos indicus* x *Bos taurus* crosses.

Results from this study indicate favorable potential for use of *Bos indicus* germplasm in designed crossbreeding programs in the more temperate regions of the U.S. It is important to note that these results are applicable to cow-calf production systems to a weaning endpoint but may change if evaluated on a slaughter basis from any price differential in carcass value from *Bos indicus-X* relative to *Bos taurus-X* calves or differential feedlot performance between the two.

Table 1—Output/input of differences among F_1 cows of diverse biological type

Item	Overall mean	Breed group[a]			
		HA-X	Bm-X	Sw-X	Pz-X
Progeny (126 days)					
Wt gain, lb	284.3	92	108	103	99
Energy consumed, Mcal ME[a]	592.2	112	92	94	102
Dams (126 days)					
Milk prod., lb/d	15.5	90	105	101	103
Cow wt, lb	1,236	98	105	97	100
Fat probe, in	.31	91	102	112	95
Energy consumed, Mcal ME	3,292	93	106	97	104
Efficiency (126 days)					
Progeny gain, lb/Mcal ME intake by cow and calf	.073	95	104	106	95

[a] Ratio percentages computed relative to overall mean where HA-X = Hereford or Angus, Bm-X = Brahman, Sw-X = Sahiwal and Pz-X = Pinzgauer sired F1 crosses out of Hereford and Angus dams.

Characteristics of Diverse Breeds in Cycle IV of the Cattle Germplasm Evaluation Program

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Introduction

Breed differences in performance characteristics are an important genetic resource for improving efficiency of beef production. Diverse breeds are required to exploit heterosis and complementarity through crossbreeding and new composite breeds and to match genetic potential with diverse markets, feed resources and climates. This report presents preliminary results from an ongoing study at the Roman L. Hruska U.S. Meat Animal Research Center (MARC) to characterize breeds of cattle representing diverse biological types for bioeconomic traits that influence quantity and value of production.

Experimental Procedure

Table 1 shows the mating plan for Cycles I, II, III, and IV of the Germplasm Evaluation (GPE) Program. Each cycle consisted of mating Hereford and Angus cows by artificial insemination (AI) to sires of diverse breeds. Semen from the same Hereford and Angus bulls has been used in all four cycles to produce control Hereford-Angus (original HAx, sires born 1963-70) reciprocal and Angus cows by artificial insemination (AI) to sires of diverse breeds. Semen from the same Hereford and Angus bulls has been used in all four cycles to produce control Hereford-Angus (original HAx, sires born 1963-70) reciprocal crosses in each cycle. In Cycle IV, new samples of Hereford and Angus (current HAx, sires born 1982-84) bulls were added to evaluate genetic trends within these breeds. In Cycle IV, semen from 14 original control Angus, 11 original control Hereford, 30 current Angus, 32 current Hereford (14 horned and 18 polled), 29 Longhorn, 24 Piedmontese, 31 Charolais, 29 Salers, 31 Galloway, 22 Nellore, and 26 Shorthorn bulls was used by AI to produce a total of about 200 calves per sire breed in five calf crops (1986-1990). Following an AI period of about 45 days, one or two bulls each of Angus, Hereford, Charolais, Gelbvieh, and Pinzgauer breeds were used each year by natural service in single-sire breeding pastures for about 21 days. These breeds were used in clean-up matings to increase ties to previous cycles and facilitate pooling of results over all four cycles.

Calves were born in the spring, beginning in late March and ending in late May. Calves were weighed, tattooed, and tagged for identification. Male calves were castrated within 24 hr of birth. Calves were creep fed whole oats from mid July until weaning in early October. Calves averaged about 170 days of age at weaning. Following a postweaning adjustment period of about 28 days, steers were penned and fed separately by sire breed for about 200 to 263 days. A growing diet (dry matter basis) containing 66% corn silage, 22% corn, and 12% supplement was fed until steers weighed about 700 lb. A finishing diet containing 25% corn silage, 70% corn, and 5% supplement was fed from about 700 lb to slaughter. Representative samples of steers were slaughtered serially in 4 slaughter groups spanning at least 63 days. The steers were slaughtered in a commercial packing plant, and hot carcass wt were obtained and used to compute dressing percentages (100 X carcass wt/final live wt). After a 24-hr chill, USDA yield grade (fat thickness, ribeye area, estimated percentage of kidney fat) and quality grade (marbling, maturity) data were obtained. The right side of each carcass was returned to the meat laboratory at MARC and fabricated into boneless, retail cuts trimmed to .3 in fat thickness and weighed. Retail cuts were then trimmed free of fat (.0 in) and reweighed. Retail product, including all steaks, roasts and lean trim (trimmed to 20% fat basis) from the right side, was doubled to estimate retail product yield from the carcass in terms of wt and as a percentage of cold carcass wt. Warner-Bratzler shear determinations of tenderness were taken on cooked rib steaks from each carcass following standard procedures. Palatability characteristics of cooked steaks were evaluated by a trained sensory panel. Steaks for shear and sensory panel evaluation were cooked at 166°C to 70°C (medium).

All F_1 females produced were retained to evaluate growth, age at puberty, reproduction and maternal performance through mature ages. Heifers were fed in a drylot from weaning to about 370 days of age on a diet containing 54% corn silage, 42% alfalfa haylage, and 4% supplement until January, and 45% corn silage, 54% alfalfa haylage and 1% supplement until they were moved to grass in the spring. Estrus was checked visually twice daily from an avg age of about 250 days until the middle of the breeding season at about 420 days of age.

Date at puberty was defined as date of first observed estrus confirmed by a subsequent estrus observed within 45 days. Females were bred by natural service to Red Poll sires to produce their first calves as 2-year-olds andsubsequently to Simmental sires through mature ages. Preweaning management was the same as that described above for F_1 crosses, except that progeny of F_1 dams were not creep fed.

Results

Breed cross means averaged over Hereford and Angus dams are presented in Table 2 for calving difficulty, birth wt, calf survival, and 200-day wt for the five calf crops produced in Cycle IV of the program. F_1 cross progeny by current Hereford and Angus sires were heavier at birth (5.8 lb) and weaning (29 lb) than F_1 cross progeny by original Hereford and Angus sires, indicating that significant genetic change for growth rate accrued between the late 1960's and the early 1980's in response to selection emphasis by seedstock breeders of both of these breeds.

Relative to original Hereford-Angus crosses, the results for birth wt and 200-day weaning wt of Charolais and Gelbvieh crosses were consistent with those observed in previous cycles. Relative to original Hereford-Angus crosses, the Pinzgauer sampled in Cycle IV were heavier at birth, weaning and as yearlings than the sample included in Cycle III of the program. Weaning wt of Longhorn crosses were the lightest. Galloway were similar in weaning wt to Hereford-Angus crosses by original sires. Weaning wt of Shorthorn crosses were similar to Salers, Pinzgauer,

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Gelbvieh, current Hereford-Angus and approached the heaviest breed groups, Nellore and Charolais.

Breed cross means for final wt and carcass and meat traits are presented in Table 3 for steers from the first four of five calf crops to be produced. Differences among breed crosses for final wt (419 days) correspond closely to those observed for weaning wt, except that Nellore crosses were lighter after the postweaning period which included the winter months, indicating that Nellore crosses perform better during the summer months than during the winter months. Progeny of current Hereford and Angus sires were significantly heavier than progeny of original sires; however, carcass composition and marbling score were similar. Breed crosses that excelled in marbling score (Choice quality grade, Shorthorn, Angus-Hereford crosses) had the lowest percentage of retail product. Breed crosses that had the highest percentage of retail product (Piedmontese, Charolais, Gelbvieh, and Salers) had the lowest marbling score (Select quality grade).

Carcasses from Galloway and Longhorn crosses had higher percentage retail product, but were lighter in wt than Hereford-Angus crosses. Piedmontese crosses excelled in carcass composition. Although Piedmontese crosses were comparable to original Hereford-Angus crosses in final wt, they ranked second to Charolais in wt of totally trimmed (.0 in) retail product due to a higher dressing percentage and a significantly higher retail product percentage than other breeds. Salers crosses were intermediate in growth rate to weaning and yearling ages. Marbling score was low but retail product yield as a percentage of carcass wt was high in Salers crosses. Salers, Gelbvieh and Nellore crosses were comparable in lean growth potential as reflected in wt of retail product at 419 days of age.

Breed crosses ranked differently for marbling score than they did for tenderness (Shear force). Shorthorn crosses excelled in marbling score but shear force values were comparable to those of other *Bos taurus* sired breed groups with lower levels of marbling. Marbling score was relatively low, but steaks were relatively tender (low shear force values) in Piedmontese crosses, whereas Salers also had low marbling but relatively high Shear force values. Marbling score was also low in Nellore crosses and their shear be higher than all other crosses. Steaks from Nellore crosses, like *Bos indicus* breeds evaluated earlier (Brahman and Sahiwal), were less tender than those from *Bos taurus* sire breeds.

Breed cross means for 400-day wt, 550-day wt, puberty traits and conception rates of heifers are presented in Table 4. Age at puberty is reported only for females resulting from AI matings (progeny of sires used in clean up matings at the end of the breeding season are not included). Breed cross differences for 400- and 550-day wt in heifers (Table 4) correspond closely to those for final wt (417 days) in steers. Nellore crosses, like other *Bos indicus* breeds (Brahman and Sahiwal) evaluated earlier in Cycle III of the program, were oldest at puberty. In spite of older age at puberty, their conception rate as yearlings was above average. Piedmontese crosses, like other breeds that have been selected for milk production, reached puberty at young ages. In previous cycles of the GPE Program, breeds which have had a history of selection for milk production (e.g., Simmental, Gelbvieh, Brown Swiss, Pinzgauer) reached puberty at younger ages than breeds that had not been selected for milk production (e.g., Charolais, Limousin, Chianina, Hereford-Angus). The relationship between age at puberty and conception rate was relatively low; however, because a high percentage of the heifers in all breed groups were cycling by the time the breeding season was initiated.

Means for calf crop percentage, calving ease, birth and weaning wt of progeny are shown in Table 5. Results are presented from four calf crops of females born in 1986, three calf crops from females born in 1987 and two calf crops from females born in 1988, and one calf crop from females born in 1989. It is emphasized that these results are preliminary, including females from the first four of five calf crops to be produced in Cycle IV. Data willobtained on the females for an additional four calf crops. Means for traits such as conception rate, percentage calf crop born and weaned, and percentage calvings unassisted may change as additional data accumulate because they have larger experimental errors due to their all or none (calf or no calf) nature.

Birth wt of progeny and calving assistance were low for Nellore and Longhorn crosses. Even though Salers cross progeny had high birth wt, calving assistance tended to be low for Salers crosses. Birth wt of progeny of Hereford-Angus cross females by current sires were heavier than those by original sires but calving assistance was similar. Progeny of Salers, Nellore, Shorthorn, Pinzgauer, and Gelbvieh crosses were heavier at weaning than those of current Hereford-Angus crosses and Piedmontese crosses, which were in turn heavier than those of original Hereford-Angus crosses or Longhorn and Galloway crosses.

Table 1—Sire breeds used in Germplasm Evaluation Program

Cycle I (1970-72)	Cycle II (1973-74)	Cycle III (1975-76)	Cycle IV (1986-90)
F_1 crosses from Hereford or Angus dams (Phase 2)			
Hereford	Hereford	Hereford	Hereford[a]
Angus	Angus	Angus	Angus[a]
Jersey	Red Poll	Brahman	Longhorn
S. Devon	Brown Swiss	Sahiwal	Salers
Limousin	Gelbvieh	Pinzgauer	Galloway
Simmental	Maine Anjou	Tarentaise	Nellore
Charolais	Chianina		Shorthorn
			Piedmontese
			Charolais
			Gelbvieh
			Pinzgauer
3-way crosses out of F_1 dams (Phase 3)			
Hereford	Hereford		
Angus	Angus		
Brahman	Brangus		
Devon	Santa Gertrudis		
Holstein			

[a] Hereford and Angus sires, originally sampled in 1969, 1970 and 1971, have been used throughout the program. In Cycle IV, a new sample of Hereford and Angus sires produced after 1982 were used and compared to the original Hereford and Angus sires.

Table 2—Breed cross differences in preweaning traits, Cycle IV - Phase 2 calves

Breed group of calf	No. calves Born	No. calves Wnd.	Calvings unassisted %	Birth wt lb	Calf survival %	200-day wt Units lb	200-day wt Ratio %
Original HA-X	192	185	94.4	77.9	96.3	465	94.2
Current HA-X	100	94	95.2	83.7	92.9	494	100.0
Charolais-X	203	184	91.2	89.1	90.6	505	102.2
Gelbvieh-X	226	211	97.7	87.8	93.4	500	101.3
Pinzgauer-X	226	213	95.9	88.4	95.4	497	100.4
Shorthorn-X	181	170	99.9	86.1	93.4	496	100.4
Galloway-X	173	164	98.0	80.1	94.6	465	94.2
Longhorn-X	202	187	99.7	69.1	92.4	441	89.3
Nellore-X	197	184	94.6	89.3	93.1	509	103.1
Piedmontese-X	202	188	94.7	83.6	92.6	489	99.1
Salers-X	189	176	97.5	84.3	93.6	499	100.9

Table 3—Breed cross differences in final weight and carcass traits of steers, Cycle IV - Phase 2

Breed group of steer	No.	Final wt. lb	Dress. pct. %	Marb-ling score	Shear force lb	Fat thick-ness in	Rib eye area sq in	Retail product .3 in trim %	Retail product .0 in trim %	Retail product .3 in trim lb	Retail product .0 in trim lb
Orig. HA-X	80	1116	62.0	531	11.8	.65	11.22	67.8	62.1	447	409
Cur. HA-X	34	1205	62.1	523	12.3	.61	11.18	68.2	62.5	487	445
Charolais-X	86	1235	61.8	496	13.0	.37	12.18	71.2	66.0	522	483
Gelbvieh-X	105	1188	61.8	498	12.5	.36	12.06	71.6	66.4	503	466
Pinzgauer-X	96	1167	61.0	525	11.2	.40	11.40	70.4	65.1	483	446
Shorthorn-X	95	1202	61.9	548	12.9	.47	11.08	68.0	62.5	484	444
Galloway-X	75	1077	62.2	512	12.8	.46	11.28	70.7	65.2	453	417
Longhorn-X	92	1006	61.5	508	13.4	.35	10.74	70.4	65.1	418	386
Nellore-X	97	1143	64.2	486	15.8	.47	11.35	70.2	64.7	495	455
Piedmontese-X	80	1130	63.6	492	11.9	.29	13.19	74.4	69.8	512	480
Salers-X	77	1188	62.3	496	14.0	.38	11.94	71.0	65.7	503	466

[a] Means for wt and carcass traits at avg slaughter age of 419 days.

[b] Marbling score: Slight = 400 to 499, small = 500 to 599, etc. Small meets minimal requirements for USDA Choice quality grade.

Table 4—Breed cross differences in growth and puberty traits, Cycle IV - Phase 2 heifers

Breed group of heifer	No.	400-day wt lb	550-day wt lb	Puberty expressed[a] %	Age at puberty[ab] act. days	Age at puberty[ab] adj. days	Preg. rate[ab] %
Original HA-X	88	783	874	88.0	343	351	93.3
Current HA-X	55	820	926	91.2	349	355	82.9
Charolais-X	85	828	956	92.2	344	349	82.0
Gelbvieh-X	103	799	932				
Pinzgauer-X	97	821	945				
Shorthorn-X	73	842	941	90.1	342	348	91.8
Galloway-X	76	764	853	89.2	346	353	83.3
Longhorn-X	81	705	814	77.1	344	357	94.3
Nellore-X	82	799	922	51.0	370	395	91.6
Piedmontese-X	91	777	881	91.0	333	339	98.0
Salers-X	90	835	945	95.7	353	356	91.9

[a] Results for puberty traits and conception rate are for AI sired progeny only. Clean-up sired progeny are not included in means reported.
[b] Actual age at puberty for the heifers expressing puberty (ranging from 51.0 to 95.7%) and mean age at puberty adjusted to 100 percent puberty basis.

Table 5—Breed cross differences in reproductive and maternal traits, Cycle IV - Phase 3 - calves born in 1988-1991[a]

Breed group of dam	No. cows exposed	Calf crop born %	Calf crop weaned %	Calvings unassisted %	Birth wt lb	200 day wt units lb	200 day wt ratio %
Original HA-X	389	91.0	86.1	77.6	82.4	449	93.5
Current HA-X	206	88.5	82.9	80.0	86.7	480	100.0
Charolais-X	183	89.7	83.9	78.0	88.6	484	100.8
Gelbvieh-X	201	81.6	77.6	81.2	84.2	494	102.9
Pinzgauer-X	206	90.4	83.8	76.0	87.4	491	102.3
Shorthorn-X	119	92.4	87.5	80.7	90.6	504	105.0
Galloway-X	172	85.2	80.1	83.0	79.9	438	91.2
Longhorn-X	191	94.4	85.9	88.2	78.5	446	92.9
Nellore-X	183	92.3	83.9	94.6	74.8	501	104.4
Piedmontese-X	215	93.0	86.7	72.9	84.2	473	98.5
Salers-X	180	90.3	86.3	87.3	86.5	506	105.4

[a] Data were analyzed for 957 matings of F_1 females to Red Poll bulls to produce first calves at 2 years of age and 1,525 matings of F_1 females to Simmental bulls to produce subsequent calves at 3, 4, and 5 years of age.

Genes of the Major Histocompatibility Complex in Cattle

Roger T. Stone and Noelle E. Muggli-Cockett[1]

Introduction

The search for simple genetic traits that can be used as markers to predict variation in more complex genetic traits has been ongoing for several decades. For a given gene to be useful as a marker, it must have multiple forms, alleles, that are readily identifiable. Also, the frequency for the different alleles of the gene in a population must be such that most animals have two forms of the gene instead of one, otherwise statistical analysis is difficult. Only a few relationships between markers and production traits reported thus far have been utilized in production practices, presumably because of economic considerations. However, inexpensive tests to predict the genetic potential of individual breeding animals for multiple traits having strong genetic influence would likely gain acceptance and benefit the livestock industry. Initial studies in this area attempted to use blood types or blood protein variants as markers. These markers (proteins) had no apparent relationship to the variation in economic traits themselves, nor was there any knowledge of their proximity or linkage to other genes controlling economically important traits.

Recombinant DNA technology has made the idea of marker assisted selection more feasible because it is now possible to isolate and study target genes that are known to, or are likely to, impact important traits such as disease resistance, reproduction, or growth. Also, as a result this technology it is possible to identify short stretches of DNA that are inherited in many allelic forms and are distributed randomly over the entire genome. This new kind of marker will allow for the identification of additional target genes through mapping studies and may be useful as tags to follow the inheritance of alleles of target genes which have limited numbers of allelic forms. Important advances in the statistical analysis of this type of genetic data have been made recently. All things considered, there is reason to be optimistic that in the next few years genetic markers can be useful as tools in selection programs.

In the past few years, we have concentrated on a complex cluster of genes that are known to be involved in the immune response. These genes are the major histocompatibility complex (MHC) which are referred to as BoLA in cattle. The MHC genes produce two types of protein products. The class I proteins are present on the surface of most cells and function in the rejection of foreign cells such as transplants, tumors, or virus-infected cells. The class II proteins occur in cells of the immune system and are important in antibody formation. Antibody formation is initiated when specialized cells in the immune system take up foreign proteins, antigens, and cleave them into small fragments that are bound by the class II proteins. The binding of antigen fragments from vaccines or pathogens is a critical step in antibody formation which makes the class II genes good candidates to be markers for disease resistance or overall immunity. There are several class II genes and most of these genes have many variants, alleles. Experiments utilizing inbred strains of mice have shown that different class II alleles recognize different fragments of an antigen with differing efficiencies. Some alleles did not recognize any fragments of a rather large antigen which leads to a lack of antibody production. This result may provide an experimental basis for individual variations in antibody production observed in laboratory and domestic animals. We have isolated and characterized some of these class II genes from cattle and have used parts of these genes as probes to follow the inheritance of their allelic forms. We have also analyzed the association of several alleles with growth traits and antibody titers to vaccines.

Procedure

Bovine class II MHC genes were isolated from a library made by placing DNA fragments representing all of the bovine genome into a suitable cloning vector and using corresponding human genes to identify clones containing the genes of interest. The DNA sequences were determined for parts of the genes and the identity of the genes confirmed by comparing the sequences to previously sequenced human genes.

Blood samples from straightbred Hereford and Angus cattle were used for DNA preparations. To determine the alleles for the MHC genes, DNA was digested with restriction enzymes and the digested fragments separated according to size by electrophoresis. The DNA fragments corresponding to the various class II genes were detected by reacting them with specific parts (probes) of the genes that had been isolated and made radioactive. The pattern of fragment sizes defined allelic forms of each gene. Statistical analysis was performed to determine whether any allele of MHC genes in these herds had an association with birth weight, weaning weight, or yearling weight. Antibody titers to BVD and BRSV were determined by Dr. Clayton Kelling (Department of Veterinary Science, University of Nebraska, Lincoln) in response to vaccination and analyzed for associations with MHC type.

Results

Three of the genes isolated corresponded to the human DRB group of genes. Sequence analysis and other types of analysis showed that only one of these genes was functional. This result suggested that any statistical analysis should be based on the alleles of this functional gene and not those of the two nonfunctional genes. A representative of another class II gene family, DQB, was isolated and shown to have several alleles in cattle. A fourth gene, DIB, was found to be considerably different from any previously isolated genes. Further analysis showed that this gene was present only in cattle and other members of the cattle (Bovidae) family. From our data and data from other laboratories, it appears that members of the cattle family have a unique second cluster of at least three class II genes located a considerable distance from the traditional tightly linked MHC class I, DR and DQ gene cluster. These genes may provide other laboratories with the genetic tools to trace the evolution of the cattle family and it provides another set of markers to analyze for associations with production traits that may be controlled by genes located on this region of bovine chromosome 23.

Statistically significant associations of MHC allelic types were obtained with weaning weight, adjusted 205-day weight, preweaning average daily gain, BVDV antibody titers 60 days after vaccination, and BRSV antibody titers 30 days of age and after weaning. These associations are encouraging, but have to be interpreted cautiously. Several associations with

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MHC type alleles have been published, but a portion of these have not been confirmed in further experiments. Typically, these experiments utilize a small number of sires and it is not uncommon to obtain an association in the offspring from only one sire in a study. This suggests that the genes controlling the traits of interest may be separated from the marker genes by a considerable distance, which means that arrangement of alleles for marker genes and those of the genes of interest are not the same across sires because of recombination. All of the growth traits mentioned above were associated only with alleles of DIB and not those of DRB or DQB, which are close together (Table 1). The alleles of DIB recombine with the DRB and DQB 20% of the time, i.e., DIB is separated by a distance of 20 units. It may be important that the association of DIB with growth parameters was observed in both Angus sires in this study. The possibility that DIB is closer to a gene(s) that influences growth than are DRB/DQB, merits further investigation.

Perhaps the most important finding during the course of these studies is that there are many more alleles of the class II MHC genes than initially supposed. Based on DNA sequence data from this and other laboratories there are at least 20 forms, alleles, of both DRB and DQB. This could explain some of the inconsistency in past analysis for associations, because not all of the alleles were being detected. Future studies will need to account for all alleles and will need to concentrate on well defined populations containing a minimum number of alleles. Albeit, based on the number of studies showing associations of the MHC types with growth, reproduction, and immune traits, there is reason to believe that there are genes other than the MHC genes on chromosome 23 in cattle that influence economically important traits. Efforts to map this chromosome may lead to the location of these genes, or at least markers, close enough to these genes to be used in genetic and/or selection studies.

Table 1—Least squares means of weight traits associated with DIB alleles

Sire DIB allele	Weaning weight (lb)	205 d weight (lb)	Preweaning ADG (lb/day)
Angus 86-1			
Allele 1	310.2	423.5	1.69
Allele 2	322.5	442.2	1.76
Angus 86-2			
Allele 1	316.6	424.8	1.69
Allele 2	357.5	472.3	1.91

Effect of Marbling on Variation and Change in Beef Tenderness in *Bos Taurus* and *Bos Indicus* Crosses

Robert M. Koch, John D. Crouse, Michael E. Dikeman, Larry V. Cundiff, and Keith E. Gregory[1]

Introduction

Today's diet conscious consumers continue to desire flavorful, tender meat, but increasingly avoid excess fat. Differences in USDA quality grades within similar aged cattle are determined primarily by differences in marbling scores which tend to be associated with overall fatness in beef carcasses. Previous studies (Cundiff et al., 1988) demonstrated an antagonism between lean yield in carcasses and degree of marbling associated with higher quality grades. Breeds that rank highest for retail product percentage rank lowest for marbling. High negative genetic correlations have been found within breeds between marbling and retail product percentage. Thus, only limited opportunity exists for genetically increasing marbling without increasing fat trim and reducing retail product percentage. Nevertheless, there is a large amount of variation in palatability characteristics among animals with the same degree of marbling, suggesting the importance of factors other than marbling have a large impact on eating qualities. Concern with the antagonism between marbling and retail product percentage is justified to the extent that a certain amount of marbling is required to ensure papatability of the retail product. This report summarizes the sensory tenderness evaluations associated with marbling scores from steer carcasses produced in the Germplasm Evaluation Program at the U.S. Meat Animal Research Center.

Procedure

Sensory panel tenderness scores (SPT) from 1221 animals produced by 16 breeds of sire mated to Angus and Hereford cows in Cycles I, II and III were analyzed. Sire breeds were grouped by species; 1) *Bos taurus* (Angus, Brown Swiss, Charolais, Chianina, Gelbvieh, Hereford, Jersey, Limousin, Maine Anjou, Pinzgauer, Red Poll, Simmental, South Devon and Tarentaise) or 2) *Bos indicus* (Brahman and Sahiwal).

Steers were slaughtered at a commercial packing plant. After a 48-hr chill, carcasses were evaluated for conformation and maturity. Marbling, lean color, texture and firmness were evaluated in the longissimus muscle interface. USDA quality grade (USDA, 1975) was determined by representatives of the U.S. Meat Animal Research Center, of the Standardization Branch, Agricultural Marketing Service, USDA, and of Kansas State University. The right side of each carcass was transported to Kansas State University for detailed cutout and taste panel evaluation. Steaks from the longissimus muscle at the 10th rib were cooked at 177 c to an internal temperature of 65 C and evaluated by an experienced taste panel for tenderness, flavor and juiciness. Sensory scores ranged from 9 = extremely tender, 5 = acceptable, 1 = extremely tough. Longissimus muscle fat percentage was determined by chemical analyses of the 12th rib steak.

Results

Average values of longissimus muscle fat percentage, sensory panel tenderness and standard deviations of sensory panel scores within each degree of marbling for *Bos taurus* and *Bos indicus* groups are shown in Table 1. Trends are illustrated in Figure 1. Average taste panel scores improved as marbling increased when comparisons were at the same age, but the change was relatively small. Variation as measured by the standard deviation within marbling degrees tended to be greater at low levels of marbling than at higher levels. This in turn increased the risk of at least some steaks having less than acceptable tenderness (see SPT<5, Table 1 and Figure 1). In *Bos taurus* sired cattle with a slight degree of marbling (USDA Select), 3% of the steaks were scored as less than acceptable in tenderness. In *Bos taurus* sired cattle with moderate or greater degrees of marbling (USDA High Choice or Prime), 0% of the steaks were scored as less than acceptable (i.e., 100% with scores > 5). Sensory panel scores for steaks from *Bos indicus* sired steers were lower for tenderness and the percentage less than acceptable was higher than for those from *Bos taurus* sired steers, even at the same degree of marbling.

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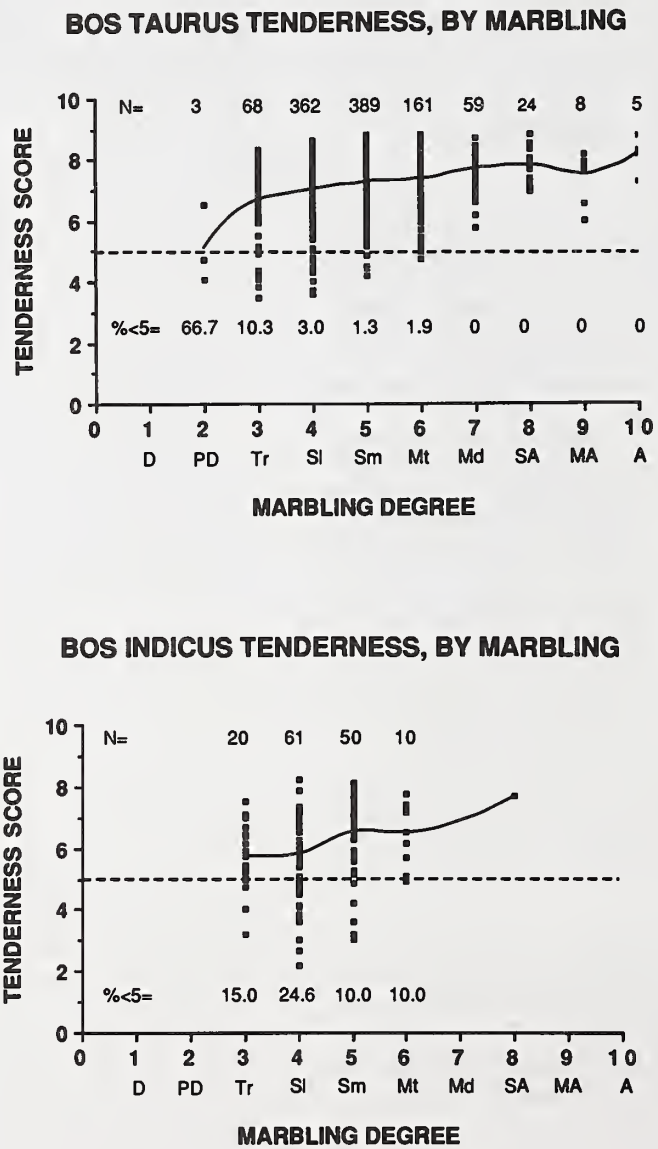
Table 1—Sensory panel tenderness and marbling scores[a]

Marbling	*Bos taurus* sired					*Bos indicus* sired			
	LMF%	No.	SPT	SD	SPT<5	N	SPT	SD	SPT<5
P. devoid	2.1	3	5.1	1.2	66.7				
Traces	2.7	68	6.7	1.1	10.3	20	5.7	1.1	15.0
Slight	3.7	362	7.0	.9	3.0	61	5.8	1.3	24.6
Small	5.0	389	.3	.8	1.3	50	6.5	1.2	10.0
Modest	6.6	161	7.4	.8	1.9	10	6.5	1.0	10.0
Moderate	8.0	59	7.7	.6	0				
Sl. abundant	9.0	24	7.8	.5	0	1	7.7	0	0
Md. abundant	11.1	8	7.4	.8	0				
Abundant	13.6	5	8.1	.5	0				

[a] LMF% = longissimus muscle fat percentage; SPT = sensory panel tenderness scores with 9 = extremely tender, 5 = acceptable, and 1 = extremely tough; SD = standard deviation with marbling degree; SPT<5 = percentage sensory scores less than acceptable.

Figure 1. Effects of marbling on mean sensory panel tenderness scores in *Bos taurus* and *Bos indicus* crosses.



Genotype-environment Interactions for Reproduction and Maternal Performance of *Bos indicus* and *Bos taurus* Crosses in Nebraska and Florida

Larry V. Cundiff, Timothy A. Olson, K. Euclides Filho, M. Koger, W. T. Butts and, Keith E. Gregory[1]

Introduction

In the U.S., cattle of diverse breeds and crosses are maintained in diverse climatic environments ranging from the temperate-continental conditions of the North Central region, characterized by wide fluctuations in temperate from winter to summer, to subtropical conditions of the Southeastern region, characterized by relatively moderate winter temperatures but high temperatures and humidity in the summer mo. The genetic range is spanned by *Bos indicus* (humped cattle) breeds, that originally evolved under tropical conditions of India and Pakistan, and by *Bos taurus* (nonhumped) breeds, that originally evolved under temperate conditions of continental Europe and the British Isles. The present experiment was conducted to investigate genotype-environment interaction for reproduction and maternal performance of *Bos indicus* x *Bos taurus* (Bi X Bt) F_1 cross cows compared to *Bos taurus* x *Bos taurus* (Bt X Bt) F_1 cross cows in a temperate (Nebraska) and a subtropical (Florida) environment. The term genotype-environment interaction applies when differences between genotypes (e.g., breeds, lines within breeds, sire progeny groups) found in one environment (e.g., climate, diet, location) change in magnitude or even rank when compared in other environments.

Procedure

This study included wt and survival data on 2,744 crossbred calves from Bt X Bt F_1 dams including Hereford X Angus and Angus X Hereford (HAX), and Pinzgauer X Angus and Pinzgauer X Hereford (PzX); and Bi X Bt F_1 dams including Brahman X Angus and Brahman X Hereford (BmX), and Sahiwal X Angus and Sahiwal X Brahman (SwX) crosses. These dams were produced in the spring of 1975 and 1976 in Cycle III of the Germplasm Evaluation (GPE) Program at MARC. The cows were progeny of Angus and Hereford dams that had been artificially inseminated to 13 Hereford, 16 Angus, 9 Pinzgauer, 17 Brahman and 6 Sahiwal (semen imported from Australia) sires.

Shortly after weaning in November of each yr, about 33% of each group of paternal half-sib heifers were transferred to the Subtropical Agricultural Research Station (ARS-USDA) at Brooksville, Florida; the other 67% remained at MARC. The heifers were maintained under standard management practices at each location. At MARC, heifers were developed on mixed silage based diets (corn silage, alfalfa haylage, and soybean meal plus antibiotics) until they were moved to cool-season pasture in April of each yr or warm season pasture from mid- to late summer. At Brooksville, heifers were maintained on permanent grass pastures (mainly Pensacola bashaiagrass) and supplementally fed a 20% range pellet, molasses and grass hay from November to April. At subsequent ages, the cows were maintained on improved pastures through November, beginning in mid-March in Florida and mid-April in Nebraska. Legume or grass hay and protein supplement were fed during the winter mo at both locations.

Females at both locations were pasture mated to Red Poll bulls produced at MARC for their first calves and to upgraded (7/8) Simmental bulls produced at MARC for their second and all subsequent calves. At Nebraska, heifers were mated to produce their first calves at 2 yr of age. Due to use of earlier breeding seasons at Florida (March 15 to May 20) than at MARC (May 15 to July 20), heifers born in Nebraska and transferred to Florida did not reach puberty early enough for breeding to calve as 2-yr-olds and were bred to calves as they approached 3 yr of age. Calves were born in March and April in Nebraska and from late December through February in Florida. Calves were weaned in October in Nebraska at an avg age of 201 days and in August in Florida at an avg age of 218 days.

Data for pregnancy rate, birth wt, rate of unassisted calving, survival to weaning, age at weaning, preweaning growth rate, and weaning wt were analyzed using appropriate least squares procedures to estimate effects of location, breed group and their interaction and to adjust for effects of yr, sex, age of dam and age of calf.

Results

Breed group by location avg are shown in Table 1. The avg pregnancy rate, based on rectal palpation at or shortly after progeny were weaned each yr, was 9% higher in Nebraska than in Florida, possibly due to the nutritional environment provided and the temperate climate in Nebraska. The pregnancy rate of Bi X Bt cows was greater than that of Bt X Bt cows by 4%. The genotype- environment interaction was important for pregnancy rate ($P = .06$). The advantage of Bi X Bt cows over Bt X Bt cows for pregnancy rate was greater in Florida (6%) than it was in Nebraska (2%).

Calving ease (unassisted calving rate) was 5% greater in Florida than in Nebraska. The increased assistance in Nebraska was primarily associated with heavier birth wt (17.5 lb) in Nebraska. Most of the advantage in calving ease was observed in first calf females producing Red Poll sired progeny. Red Poll sired calves out of first calving females had a 30% higher unassisted calving rate in Florida than in Nebraska. This large location difference was likely influenced by the age of calving difference (nearly 1 yr younger in Nebraska) and the 14 lb heavier birth wt in spite of a younger age of dam in Nebraska. The genotype-environment interaction for calving ease and birth wt ease was highly significant. The advantage of Bi X Bt cows over Bt X Bt cows for unassisted calving rate was much greater in Nebraska (13%) than in Florida (4%), reflecting a similar interaction for birth wt. Birth wt of progeny of Bi X Bt cows were 10 lb lighter than those of Bt X Bt cows in Nebraska, but only 5 lb lighter in Florida where avg birth wt were relatively light for all progeny.

Calves born in Florida had a higher survival rate than those born in Nebraska (4.5%). The advantage corresponded closely to that for unassisted calving rate. Separate analyses of survival indicated that the advantage

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for the Florida location was relatively greater for Red Poll sired progeny out of first calf females (6.4%) than for Simmental sired progeny of females calving at subsequent ages (2.9%). In Nebraska, the advantage of Bi X Bt cows in calving ease was not reflected in increased survival of progeny. The survival rate in FL for Bi x Bt was only slightly higher than that of Bt X's. Possibly the advantage in survival usually associated with calving ease was offset by increased mortality associated with reduced cold tolerance (see report on mortality and cold tolerance).

The genotype-environment interaction was highly significant for preweaning avg daily gain and weaning wt. Progeny of Bt X Bt cows gained significantly faster (10%) in the temperate environment of Nebraska than they did in Florida. On the other hand, there was no significant difference between the two locations for preweaning gain or weaning wt of progeny of Bi X Bt cows. Preweaning avg daily gain of progeny of Bi X Bt cows was significantly greater than that of progeny of Bt X Bt cows at both locations; but, due to the genotype-environment interaction, the advantage of Bi X Bt cows over Bt X Bt cows was much greater in Florida (20%) than in Nebraska (8.6%).

Weaning wt per cow exposed, reflecting differences in pregnancy rate, survival rate and weaning wt of progeny, combines the most important output components of production efficiency in cow herds. Again, genotype-environment interaction was important for weaning wt per cow exposed to breeding. The advantage of Bi X Bt cows over Bt X Bt cows was much greater in Florida (28%) than in Nebraska (5.8%).

The advantage of Bi X Bt cows over Bt X Bt cows in a subtropical environment was clearly shown in this study. Adaptation to subtropical conditions is an important component of production efficiency in the southeastern U.S. Weaning wt of calves from Bi X Bt cows also equaled or exceeded those of Bt X Bt cows under temperate conditions. Part of this advantage is likely due to greater heterosis that has been found in Bi X Bt crosses than in Bt X Bt crosses. The extra heterosis is likely attributable to greater genetic diversity between *Bos indicus* and *Bos taurus* breeds than is represented between *Bos taurus* breeds. Also, the Bi X Bt cows had lower calf birth wt and calved more easily than Bt X Bt cows. However, the advantages of Bi X Bt cows over Bt X Bt cows are tempered by increased incidence of calving difficulty when Brahman sires are mated to *Bos taurus* cows, later age at puberty in *Bos indicus* sired crosses, increased calf mortality as the proportion of *Bos indicus* inheritance increases to 50% or higher under cold calving conditions, and significantly lower meat tenderness as the proportion of *Bos indicus* inheritance increases (see other articles in this report). The most effective way to manage these tradeoffs is to use crossbreeding systems or composite populations that exploit heterosis and match genetic potential in the cow herd with the climatic feed environment. Production efficiency can be increased further by making terminal crosses to the extent possible using sire breeds that reduce the *Bos indicus* influence in slaughter progeny and increase efficiency of lean tissue gain.

Table 1—Reproduction and maternal performance of *Bos taurus* X *Bos taurus* (Bt X Bt) and *Bos indicus* X *Bos taurus* (Bi X Bt) breed crosses in Nebraska (NE) and Florida (FL)[a]

		Bt X Bt			Bi X Bt		
Trait	Location	HAX	PzX	avg	BmX	SwX	avg
Pregnancy rate, %	NE	92	93	93	94	95	95
	FL	83	80	82	86	89	88
Unassisted calving rate, %	NE	85	85	85	98	97	98
	FL	96	91	94	98	98	98
Birth wt, lb	NE	81	88	85	78	72	75
	FL	60	69	65	64	55	60
Survival rate, %	NE	93	92	93	91	92	92
	FL	98	94	96	98	97	98
Preweaning avg daily gain, lb	NE	2.02	2.17	2.09	2.34	2.21	2.27
	FL	1.83	1.96	1.90	2.34	2.24	2.29
Weaning wt per calf, lb	NE	498	536	518	560	529	544
	FL	437	473	455	553	523	538
Weaning wt per cow exposed, lb	NE	429	462	446	478	466	472
	FL	356	360	358	464	454	459

[a] HAX = Hereford X Angus and Angus X Hereford, PzX = Pinzgauer X Angus and Pinzgauer X Hereford, BmX = Brahman X Angus and Brahman X Hereford, and SwX = Sahiwal X Angus and Sahiwal X Hereford F_1 cross cows.

Gene Mapping in Cattle

Craig W. Beattie, Roger T. Stone, Michael D. Bishop, Sara L.F. Sunden, John W. Keele, and Steven M. Kappes[1]

Over the last decade, progress in molecular biologic techniques has brought the mapping of genes within the human and mouse genomes to a point where information on the location of groups of genes and additional anonymous, but unique, bits of DNA (markers) within their respective genomes can be brought to bear in developing a bovine genomic map. This is possible because of the conservation of genes, particularly those concerned with regulating important functions, between species into syntenic (single chromosome) groups.

Investment in the development of a bovine map is important for several reasons. While continued selection of desirable traits by the commercial livestock industry has made significant progress in improving moderately and highly heritable traits such as milk production, growth rate, and leanness, relatively little progress has been made in developing markers which significantly improve selection for less heritable traits or improve conception per service. Agribusiness research has also developed and provided diagnostic tests and vaccines for preventive herd health programs, yet little progress has been made in improving disease resistance while reducing costs.

To meet this challenge, genomic maps have been and are currently being developed in livestock in a number of university and commercial laboratories throughout the world. Although the development of maps for the genomes of a variety of livestock species per se will not provide additional technology for livestock improvement, they are essential for: a) providing "markers" for improved selection, and b) ultimately understanding how genes which regulate commercially important traits are expressed. Simply put, a bovine genomic map or genomic map for any economically important species is an essential starting point, not an end for development of the technology necessary to manipulate gene expression in livestock. The technological benefits and information derived from the human genome project make development of a bovine map easier. Without these benefits, mapping the genome of livestock would likely prove too expensive and long-term for the agricultural research system.

The Agricultural Research Service and others mapping, or planning to map, aspects of the bovine genome have objectives different from those of the human genome program. The initial objective in cattle is to develop and anchor 250-260 markers approximately equidistant along the genome; not to sequence the entire genome, but to provide markers for the selection of genes involved in regulating the expression of specific quantitative traits.

The two major components of the genome mapping program at the USDA-ARS MARC, Clay Center, NE are concerned with defining the location of map objects such as genes and anonymous DNA sequences and identifying which of those locations (markers) segregate to a significant extent with specific quantitative traits important to the livestock industry. Ultimately, identification of the locations or loci of genes which regulate quantitative traits (QTLs) such as marbling, meat quality, and disease resistance will allow rapid and improved selection of animals to improve these traits.

The initial objective of the genome mapping group at MARC is to simultaneously develop a genetic linkage and partial physical map of the bovine genome using identified markers. To develop a genetic linkage map, a group of anchor or index loci will serve as markers for any number of laboratories. Development of consensus index markers for similar loci is a primary focus of the genome group at MARC.

An interbreed-cross approach using families of cattle developed at MARC forms the basis for developing a genetic linkage map of the bovine genome. Since abundant polymorphisms (variations in form) within individual gene loci are generally not present within a species, a four-way cross reference population was designed to maximize this aspect of generating a map containing Type I anchor loci. Type I anchor (index) loci are evolutionarily conserved coding genes, the homologues of which are spaced at 15-25 centimorgan (CM; 15-25 million DNA bases) intervals in the human and mouse genome. The second type of linkage map takes advantage of Type II anchor loci which are species-specific DNA markers that exhibit a high degree of polymorphism. These loci include microsatellites or small repeats of particular DNA base pairs.

A physical map is based, in part, on localizing genes or known sequences of anonymous pieces of DNA directly onto an individual chromosome using a technique called *in situ* hybridization. Once identified these sites also serve as anchoring points for a map. The goal is to combine the physical and genetic linkage maps through the use of these anchor loci.

As the overall map is developed, the emphasis will shift and be expanded to include:

a) identifying markers linked to traits of economic importance,
b) characterizing selected genes at the cellular and molecular level, and
c) using highly polymorphic genetic markers in marker assisted selection programs.

The potential dissection of quantitative traits in livestock currently refractory to genetic manipulation has substantial economic implications. They include the use of genetic markers in a marker-assisted selection index program, isolation of genes regulating commercially important traits, identification of genes responsible for nondesirable traits, and improving biodiversity. The potential benefits of marker-assisted selection to the livestock producer simply in terms of cost benefit ratio from improved selection will have a proportional benefit to all livestock producers.

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Identification of Twin Pregnancies in Cattle by Ultrasonography

Sherrill E. Echternkamp and Keith E. Gregory[1]

Introduction

The increased twinning frequency (i.e., approximately 25%) in the MARC twinning project has resulted in additional management requirements to achieve survival of twin calves and timely rebreeding of their dams. Previously, neonatal survival of twin calves (i.e., within 24 hr after calving) was about 82% compared to 97% survival for single-born calves. Although twin calves are smaller at birth than single-born calves (34.3 vs 44.8 kg), the incidence of calving difficulty (dystocia) is increased in twin pregnancies (35% vs 23% for twins vs singles, respectively) due to abnormal positioning of the calves within the uterus. The dystocia further increases mortality of twin calves (27% with vs 5% without dystocia). Secondly, the production of twin births results in lower conception rates (71% for twins vs 85% for singles) and a longer postpartum infertility period (95 vs 85 days, respectively) regardless of rearing one or two calves. Precalving diagnosis of twin fetuses would enable dietary supplementation to increase maternal energy stores and increase awareness of calving difficulty. Greater obstetrical assistance at calving should reduce the effects of dystocia and nutrition on calf survival and rebreeding performance of the dam. Previously employed methods to identify twin pregnancies were: 1) measurement of estrone sulfate in maternal blood, 2) determination of number of corpora lutea and fetuses by laparoscopy, and 3) determination of fetal number by rectal palpation of the uterus. The objective of this study was to evaluate ultrasonography for diagnosing twin pregnancies in cattle. Increased prepartum nutrition and timely assistance at calving could then help reduce neonatal calf mortality and postpartum infertility associated with twin births.

Procedure

The ultrasound examination of the bovine uterus was performed between 28 and 95 days postbreeding. Initially, the uterine examinations were performed with Ausonic Micro Imager 1000 Ultrasound System equipped with a 5 MHz, angular, 90°, mechanical sector, rectal transducer. Recent examinations were performed with a 3.5 MHz convex rectal transducer (Aloka 500V). The convex transducer appears to be more durable than the mechanical sector transducer for this type of application. Cattle used in this study were from the MARC twinning project.

For the ultrasound examination the animals were restrained in a squeeze chute. The rectal transducer was coated with gel and inserted into the rectum dorsal to the cervix. The uterine body and both uterine horns were scanned systematically from posterior to anterior with small rotations of the probe from left to right. Ultrasound images of single and twin fetuses inside the lumen of the uterine horns are illustrated in Figs. 1 and 2, respectively. Tissues reflect the ultrasound waves and produce the light areas; thus the denser the tissue, the brighter the image. Liquids absorb the ultrasound waves and produce dark areas; clear fluid shows as black. Three technicians with similar experience in rectal palpation for pregnancy conducted the ultrasound evaluations[2]. Cows with fetuses less than 40 days of age and cows without an identifiable fetus were re-examined 4 wk later.

Approximately 3 mo before calving, females diagnosed with twin pregnancies were separated from the herd, housed near the calving facility and fed a high energy diet. With the approach of the calving period, these females were observed frequently for signs of dystocia and provided with the required obstetrical assistance.

Results

The twinning frequency from the fall of 1989 to the spring of 1992 was about 25% when expressed as percentage of females calving, or about 18% based on number of females inseminated (Table 1). Table 1 contains a comparison for number of fetuses by ultrasound diagnosis vs the actual number of calves born. When the number of calves born is less than the number of fetuses identified by ultrasonography, it cannot be resolved with certainty whether the reduction resulted from fetal death or a diagnostic mistake. The exception is females with fetuses at ultrasonography but nonpregnant at calving. Then it is certain that fetal death occurred. A small percentage (4-8%) of the females did abort all of their fetuses between ultrasonography and calving. The percentage of abortions was similar for females with single and twin fetuses except for the higher pregnancy failure in females with twin fetuses in the 1991 spring calving (16.8%). The 4-8% pregnancy failure for single fetuses is slightly higher than the 1-3% failure reported previously. A possible contribution of the ultrasound procedure to increased fetal death was not evaluated.

Most of the triplets were incorrectly diagnosed as twins (Table 1). The accuracy for identifying twin fetuses by ultrasonography was higher for the fall 1991 and spring 1992 calving seasons, suggesting that the skill of the technicians improved with additional experience. The main deficiency of the ultrasound procedure is to underestimate the number of fetuses in the uterus for animals with multiple fetuses. Unfortunately, the entire uterus cannot be visualized in a single ultrasound picture, so the error can result from an incomplete scan of the uterus or mistakenly identifying the two separate fetuses as the same fetus. The overall accuracy for all females evaluated was 86.1, 92.2, and 88.7%, respectively, for the three calving seasons.

As noted in Table 2, approximately one-half of the twin calves were delivered without obstetrical assistance while an additional 10% required minor assistance. Approximately one-fourth of the twin calves were positioned incorrectly within the uterus and required repositioning before delivery. These calves were subsequently assisted during delivery. As anticipated, first parity heifers tended to have a higher incidence of dystocia. Calves born without assistance had a high survival rate (i.e., low neonatal mortality; Table 2). Conversely, the highest incidence of neonatal mortality was for calves positioned abnormally in the uterus. Prior knowledge of the twin pregnancy (Table 3) did not significantly improve neonatal survival of twin calves. However, the number of incorrectly diagnosed twin pregnancies was small and most of those cows had no dystocia at calving. Survival of single-born calves (Table 4) was about 10-15% higher than for twin calves. Again, the survival of twin calves born without obstetrical complications

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[2]Ultrasound examinations were performed by MARC employees Mike Wilford, Steve Snell, and Loyal Clang.

was similar to survival of single-born calves, indicating that the increased incidence of dystocia for twins was the major cause for the lower survival of twin calves.

In summary, ultrasonography provides a quick, efficient method for diagnosis of twin pregnancies in cattle, thus, enabling provision for supplemental prepartum nutrition and greater obstetrical assistance for the females with twins. Approximately 85% of the females producing multiple births were diagnosed previously by ultrasonography. A major factor contributing to this discrepancy was failure to identify the second fetus in females with twins. Although prior knowledge of twin pregnancies did not significantly increase survival of twin calves, such information did expedite the delivery of the calves, especially when dystocia existed. Unfortunately, we do not have a solution to the abnormal positioning of the twin calves within the uterus, the major cause for the increased dystocia and reduced survival of twin calves at birth.

Table 1—Comparison of pregnancy determination by ultrasonography vs actual calving results

Ultrasound results[a]		Calving results (%)[b]							
Status	n	Triplets		Twins		Single		Open	
Spring calving 1991									
Triplets	2	0		2	(100.0)	0		0	
Twins	107	2	(1.9)	69	(64.5)	18	(16.8)	18	(16.8)
Single	324	0		16	(4.9)	296	(91.4)	12	(3.7)
Open	49	0		1	(2.0)	1	(2.0)	47	(95.9)
Total	482	2	(0.5)	88	(18.3)	315	(65.4)	77	(16.0)
Fall calving 1991									
Triplets	1	0		1	(100.0)	0		0	
Twins	78	2	(2.6)	69	(88.5)	3	(3.9)	4	(5.1)
Single	300	0		11	(3.7)	276	(92.0)	13	(4.3)
Open	111	0		1	(0.9)	3	(2.7)	107	(96.4)
Total	490	2	(0.4)	82	(16.7)	282	(57.6)	124	(25.3)
Spring calving 1992									
Triplets	1	1	(25.0)	0		2	(50.0)	1	(25.0)
Twins	98	1	(1.0)	83	(84.7)	6	(6.1)	8	(8.2)
Single	375	0		14	(3.7)	331	(88.3)	30	(8.0)
Open	70	0		0		0		70	(100.0)
Total	547	2	(0.4)	97	(17.7)	339	(62.0)	109	(19.9)

[a] Ultrasound diagnoses were conducted between 30 and 92 days of gestation.
[b] Number of females by status at calving (percentage accuracy based on ultrasound results).

Table 2—Incidence of calving difficulty (CD) and calf mortality (CM) for twin births in cattle

	First parity			Multiple parities		
Difficulty score[a]	n[b]	CD %[c]	CM %[d]	n[b]	CD %[c]	CM %[d]
1	55	51.9	5.5	264	59.2	9.8
2	13	12.3	38.5	36	8.1	8.3
3-6	9	8.5	11.1	32	7.2	0
7	2	1.9	50.0	1	0.2	0
8	27	25.5	40.7	113	25.3	23.9
Overall	106		19.8	446		12.6

[a] Calving difficulty scores are: 1=no assistance, 2=little assistance by hand, 3-6=increasing degree of difficulty, 7=caesarean, and 8=abnormal position.
[b] Total number of calves born.
[c] Distribution of calves by calving difficulty score.
[d] Percent calf mortality.

Table 3—Effect of calving difficulty and ultrasound diagnosis of twin pregnancies on the incidence of calf mortality for twin births in cattle

Parity	Difficulty score[a]	Twins diagnosed as:[b] Twins n[d]	Twins %	Single[c] n	Single[c] %	Overall n	Overall %
First	1	48	6.3	7	0	55	5.5
	2	11	36.4	2	50.0	13	38.5
	3-6	9	11.1	0	—	9	11.1
	7	0	—	2	50.0	2	50.0
	8	22	31.8	5	80.0	27	40.7
Multiple	1	215	9.3	49	12.2	264	9.8
	2	29	10.3	7	0	36	8.3
	3-6	30	0	2	0	32	0
	7	1	0	0	—	1	0
	8	101	23.8	12	25.0	113	23.9

[a] Calving difficulty scores are: 1=no assistance, 2=little assistance by hand, 3-6=increasing degree of difficulty, 7=caesarean, and 8=abnormal position.
[b] Number of fetuses within the uterus was determined by ultrasonography between 30 and 92 days of gestation.
[c] Twin pregnancies were diagnosed incorrectly as a single fetus.
[d] Total number of calves born.

Table 4—Neonatal calf survival for calves from bovine females diagnosed correctly or incorrectly by ultrasonography[a]

Parity	Twins diagnosed as: Single n	Single % survival	Twins n	Twins % survival	Single[b] n	Single[b] % survival
First	207	92.8	90	83.3	16	62.5
Multiple	729	98.5	376	87.5	70	87.1
Overall	936	97.2	466	87.4	86	82.6

[a] Results combined for three calving seasons (spring and fall calving 1991 and spring calving 1992).
[b] Twin pregnancies diagnosed incorrectly as a single fetus.

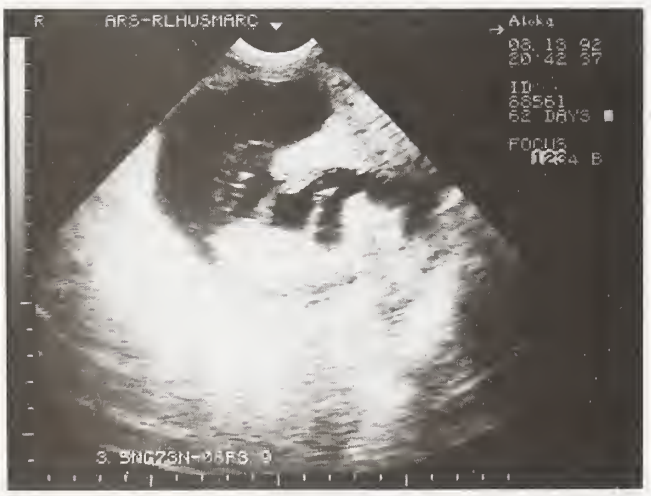

Figure 1 – Longitudinal section of a single fetus within the lumen (dark area) of the uterine horn at 62 days of gestation. The fetus is positioned on its back with the head to the right and the nose and legs pointing upward. The amniotic and allantoic fluid surrounding the fetus image as dark gray or black. The light areas above and below the fetus are uterine tissue.

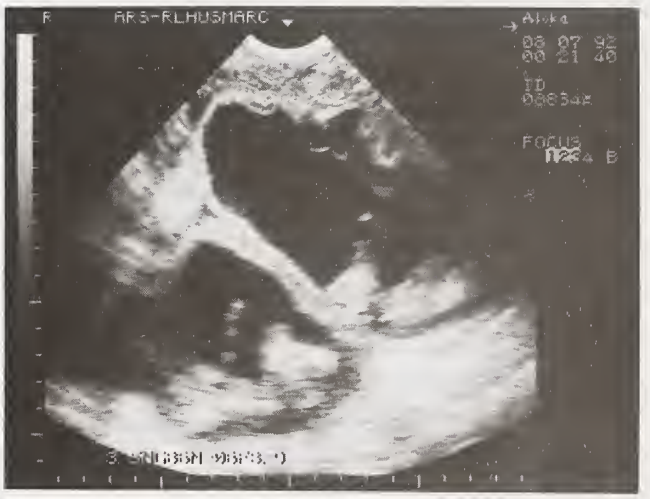

Figure 2 – Longitudinal section of twin fetuses within the lumen (dark area) of the uterus at 65 days of gestation. The fetuses are positioned on their back with the head to the center and the nose and legs pointing upward. The amniotic and allantoic fluid surrounding the fetuses image as dark gray or black andthe thin white line represents the amniotic membrane. The light areas above and below the fetuses are uterine tissue.

Hormone Profiles in Cattle Selected for Twin Ovulations and Births

Sherrill E. Echternkamp, Leon J. Spicer, and Keith E. Gregory[1]

Introduction

Follicle stimulating hormone (FSH) is secreted into the blood circulatory system by the pituitary and as the name implies stimulates growth and development of follicles within the female ovary. Thus, the administration of exogenous FSH or FSH-like substances (e.g., pregnant mare's serum gonadotropin, PMSG) to cattle has been used for the induction of multiple ovulations and, subsequently, twin or multiple births. These same substances have been used extensively for the induction of superovulation in embryo donor females. Consequently, it was speculated that cows producing twin births naturally from the spontaneous ovulation of multiple ovarian follicles would have higher circulating concentrations of FSH in their blood.

Circulating concentrations of FSH are relatively constant during the estrous cycle except at estrus (Day 0), when a preovulatory surge of FSH is released from the pituitary; a secondary release of FSH occurs about 24 hr later. Current information suggests that the preovulatory release of FSH is mediated by the release of gonadotropin-releasing hormone (GnRH) from the hypothalamus of the brain and its subsequent transport to the pituitary via the portal blood vessels. Similarly, a surge of FSH release can be induced in a dose-related response by the administration of exogenous GnRH.

Recent research has suggested that a growth factor, insulin-like growth factor-I (IGF-I), secreted predominantly by the liver can enhance the stimulatory effect of FSH on follicular growth, development, and steroidogenesis within the ovary. The significance of this finding is that increasing the amount of IGF-I to the ovary would have the same result as increasing the amount of FSH to the ovary.

Procedure

Experiment 1. To determine whether FSH and luteinizing hormone (LH) release differed between control and twin-producing cattle, 20 control and 20 twinner multiparous cows were administered two dosages (.033 mg per kg vs .22 mg GnRH per kg of body wt) of GnRH in saline 35 days apart. One-half of the cows received the low dosage and one-half the high dosage at the first treatment and vice versa at the second treatment. Cows were injected with 35 mg prostaglandin F2α (PGF; Lutalyse, Upjohn Co., Kalamazoo, MI) intramuscularly 12 to 16 days after estrus followed by a single injection of GnRH intravenously 36 hr later. Blood samples were collected at 15-min intervals via a jugular vein cannula for 4 hr before and 4 hr after GnRH injection. Concentrations of FSH, LH, and estradiol in serum from the blood samples were measured by specific radioimmunoassay (RIA) procedures.

In addition, blood samples were collected from ten twinner and ten control cows during estrus to evaluate the magnitude of the spontaneous preovulatory FSH and LH surges. The blood samples were collected from a jugular vein cannula at 4-hr intervals beginning 4 days before expected estrus to 4 days after the onset of estrus. Serum was separated from the blood sample and assayed for FSH and LH concentrations by RIA.

Experiment 2. The objective of this experiment was to compare concentrations of IGF-I and steroids in blood and ovarian follicular fluid between mature cows with and without a history for producing twin births. The estrous cycles for the two groups of cows were synchronized by a single injection of 35 mg PGF during the midluteal phase of the estrous cycle. Both ovaries and a blood sample were collected from 12 control and 14 twin-producing cows at slaughter, which occurred 48-50 hr after PGF. The diameter of all follicles on the ovarian surface greater than 4 mm in diameter were recorded and their follicular fluid was collected and stored individually. Follicular fluid from follicles 1-4 mm in diameter was collected and pooled within each cow. A blood sample was also collected at the time of PGF administration. Concentrations of IGF-I, estradiol, and progesterone were determined in samples of both follicular fluid and blood serum using specific RIA procedures.

Results

Experiment 1. Both dosages of GnRH initiated a release of FSH and LH within a few min after the injection (Figs. 1 and 2); the high dosage of GnRH produced a significantly greater release of both FSH and LH. Concentrations of FSH and LH in the blood before treatment with GnRH did not differ between control and twinner cows. Similarly, the magnitude of the GnRH-mediated FSH release (Fig. 1) did not differ between control and twinner cows for either the low or high dosage of GnRH. However, the release of LH (Fig. 2) by the high dosage of GnRH was significantly greater in twinner than in control cows, and tended to be greater in the twinner cows at the low dosage of GnRH.

A preovulatory increase (i.e., surge) in circulating concentrations of FSH (Fig. 3) and LH (Fig. 4) was detected in all animals within 0 to 8 hr after the onset of estrus. To adjust for the variation in timing of the FSH and LH surge among aminals, the hormone data were standardized to the time of the maximum LH concentrations for each animal. Magnitude of the FSH and LH surges (measured as either the highest concentration or the area of the surge) did not differ between twinner and control cows. Results obtained from these two studies suggest that FSH concentrations are not elevated in twinner cows in comparison to non-twinner cows. Thus, the increased frequency of twin ovulations in the twinner cattle herd does not appear to be associated with increased FSH secretion.

Experiment 2. Ovaries collected from twin-producing cows just before estrus had a greater number of large follicles (>4 mm) than ovaries from control cows (2.6 vs 1.9), an observation that had been anticipated with the increased frequency of twin ovulations in the twinner cows. Concentrations of IGF-I (Fig. 5) were 47% higher in blood of twinner cows than of control cows. Similarly, IGF-I concentrations were significantly higher in follicular fluid of large (>4 mm) and small (1-4 mm) follicles from twinner cows than from control cows (Fig. 5), but the differences between populations of cattle were less for small follicles. Concentrations of IGF-I in blood and follicular fluid were very similar in magnitude and were related positively (r=.88) when compared among animals, suggesting that the IGF-I in ovarian follicular fluid may be absorbed from the blood.

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Concentrations of estradiol in the follicular fluid increased as the size (i.e., diameter) of the follicle increased (Table 1). Follicular fluid estradiol concentrations were similar between twinner and control cows except for significantly higher estradiol in the second largest follicle present on the pair of ovaries (8-11.9 mm). A high concentration of estradiol in the follicular fluid of a follicle is an indication of a healthy follicle and presumably this healthy second largest follicle will produce the second ovulation for twin ovulations in the twinner cows. Progesterone concentrations in follicular fluid (Table 1) did not differ between twinner and control cows. Progesterone concentrations were related inversely to estradiol concentrations with progesterone being low in healthy ovulatory follicles and high in dying follicles with low estradiol concentrations.

Although the anticipated higher circulating concentrations of FSH were not found in the twinner cows, IGF-I concentrations were higher in twinner cows. The increased IGF-I concentrations in twinner cows should enhance the action of FSH on the ovaries and thus produce the same effects as increasing FSH concentrations to the ovaries. Additional studies are in progress to identify the role of additional growth factors (e.g., transforming growth factors α and β) and ovarian peptides (e.g., inhibin and activin) in ovarian follicular development and ovulation rate in cattle.

Table 1—Effect of follicle size on estradiol and progesterone concentrations in ovarian follicular fluid of single- and twin-producing cattle

Follicle diameter (mm)	Estradiol (ng/ml)				Progesterone (ng/ml)			
	n[a]	Single	n[a]	Twin	n[a]	Single	n[a]	Twin
< 4.0	12	12.7[b]	14	16.7[b]	12	42.2[d]	14	65.0[d]
4.0 - 7.9	5	296.2[b]	12	41.4[b]	5	111.5[d]	12	95.9
8.0 - 11.9	6	121.6[b]	9	704.2[c]	6	222.8[e]	9	90.1
≥ 12.0	12	764.2[c]	15	684.5[c]	12	37.9[d]	15	127.4[e]
Overall	35	298.7	50	361.7	35	103.6	50	94.6

[a] n = number of follicles.

[b] c Means for estradiol concentration differed among size categories ($P < .01$).

[d] e Means for progesterone concentration differed among size categories ($P < .05$).

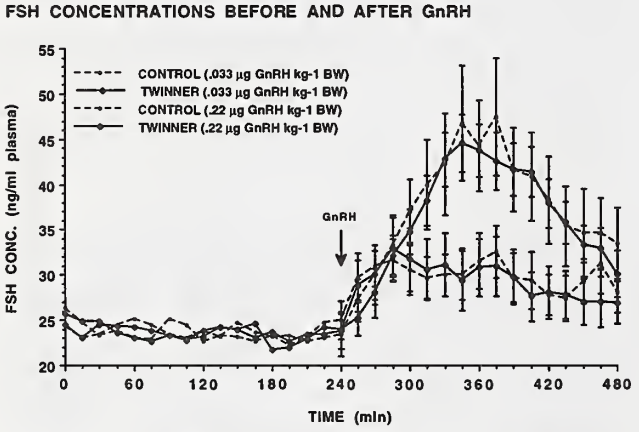

Figure 1 – Comparison of FSH releases induced with either a low (.033 mg GnRH per kg body wt) or a high (.22 mg GnRH per kg body wt) dosage of GnRH injected intravenously into cows with (twinner) and without (control) a history of twinning. The GnRH was injected 36 hr after PGF (35 mg intramuscularly) and 4 hr after initiation of the frequent blood collections (15-min intervals).

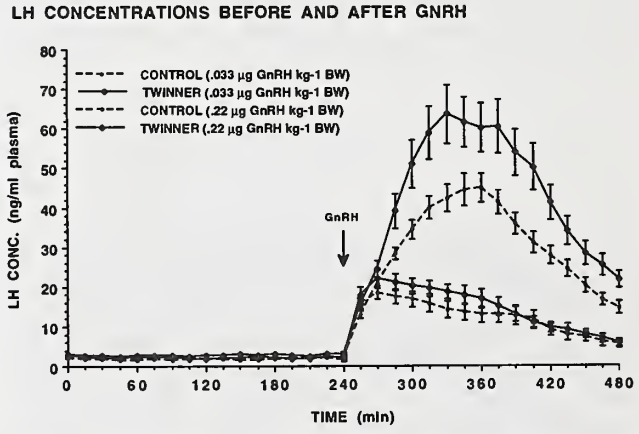

Figure 2 – Comparison of LH releases induced with either a low (.033 mg GnRH per kg body wt) or high (.22 mg GnRH per kg body wt) dosage of GnRH injected intravenously into cows with (twinner) and without (control) a history of twinning. The GnRH was injected 36 hr after PGF (35 mg intramuscularly) and 4 hr after initiation of the frequent blood collections (15-min intervals). The release of LH with the high dosage of GnRH was greater in the twinner than in control cows.

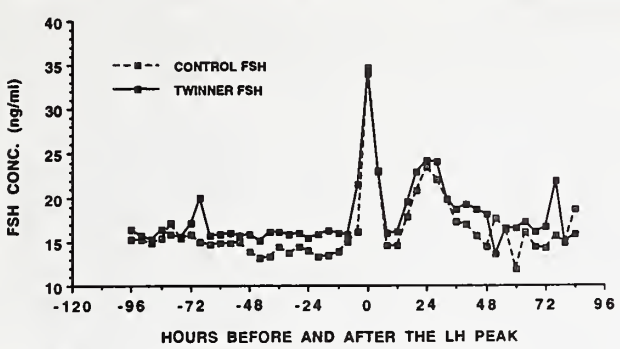

Figure 3 – Comparison of circulating FSH concentrations before, during, and after the preovulatory LH release (i.e., about 6 hr after onset of estrus) between cows with (twinner) and without (control) a history of twinning. Blood samples were collected at 4 hr intervals for 4 days before to 4 days after estrus. Data were standardized among cows by referencing sample collection time to time of maximum LH concentration (i.e., LH peak).

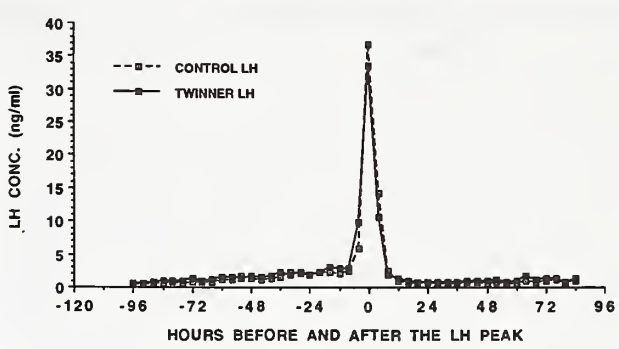

Figure 4 – Comparison of circulating LH concentrations before, during, and after the preovulatory LH release (i.e., about 6 hr after onset of estrus) between cows with (twinner) and without (control) a history of twinning. Blood samples were collected at 4 hr intervals for 4 days before to 4 days after estrus. Data were standardized among cows by referencing sample collection time to time of maximum LH concentration (i.e., LH peak).

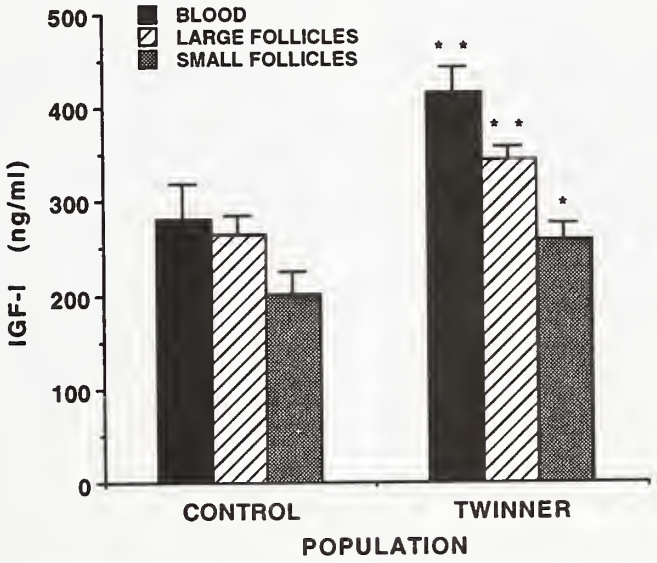

Figure 5 – Means for concentration of IGF-I in blood serum and in follicular fluid of small (1-4 mm) and large (>4 mm) ovarian follicles collected from cows with (twinner) and without (control) a history of twinning. Blood and ovaries were collected at slaughter which occurred 48-50 hr after injection of 35 mg PGF. Concentrations of IGF-I in blood and follicular fluid were significantly higher in twinner than in control cows.

Can Cattle Be Litter Bearing? Uterine Capacity in Cattle.

Sherrill E. Echternkamp[1]

Introduction

With few exceptions, the bovine female produces one ovum per estrous cycle, and consequently one offspring per year. Thus, the reproductive rate of cattle is low in comparison to other meat-producing animals, birds and fish. Studies at the University of Wisconsin in the early 1950's indicated that the frequency of twin ovulations was 10-fold greater than the frequency of twin births. However, a recent evaluation of ovulation rate in the MARC twinner herd indicated that about 50% of the twin ovulations resulted in twin births. With a twinning frequency of 25% in this population, it is suggested that the bovine female does have the capacity to gestate more than one fetus. The objective of this study was to determine the effects of multiple ovulations and/or number of fetuses within the uterus on conception rate, embryonic survival and development, maternal progesterone and estrone sulfate concentrations and placental function, and to assess uterine capacity in cattle.

Procedure

To induce multiple ovulations and, subsequently, multiple births, 283 multiparous Simmental-crossbred cows were treated with 12 mg FSH-P (follicle stimulating hormone-pituitary; Schering-Plough Corp., Kenilworth, NJ), for 4 days beginning at the midluteal phase of the estrous cycle—2 mg FSH twice daily for 2 days and 1 mg twice daily for 2 days. Estrus was synchronized by the administration of 35 mg of prostaglandin $F_{2\alpha}$ (PGF, Lutalyse, Upjohn Co., Kalamazoo, MI) intramuscularly on the morning of the 4th day of FSH treatment. Cows were pastured with fertile Gelbvieh bulls for 5 days after the FSH treatment at a ratio of 10 cows per bull and approximately 60 cows per pasture.

The number of fetuses *in utero* was estimated by ultrasonography at 45 to 60 days postmating. Pregnancy was reconfirmed at 90 to 105 days postmating by rectal palpation. Then the cows were transferred to open front buildings equipped with Calen Broadbent head gates and housed in groups of four cows of similar size and fetal number. Cows were individually fed to achieve an avg daily gain of .5 kg per day per fetus.

Blood samples (10 ml) were collected at 28-day intervals to calving; plasma was collected and stored at -20°C until assayed for progesterone and estrone sulfate (E_1SO_4) by radioimmunoassay (RIA). Progesterone was measured in a hexane extract of .1 ml of plasma using a single antibody RIA system with dextran-charcoal separation. Concentrations of E_1SO_4 were quantified in plasma that was first extracted with ether to remove the free estrone, then incubated with 100 units of sulfatase for 4 hr at 37°C. The liberated estrone was extracted with ether and assayed by a single antibody RIA using dextran-charcoal separation. For analysis of the hormone data, the last two trimesters of gestation were divided into the seven 28-day periods listed here with a range of ± 14 days: 126, 154, 182, 210, 238, 266, and 294 days.

The cows were observed frequently during calving and the calves were identified and weighed at birth. When possible, the placenta was collected, weighed and frozen for subsequent analysis.

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Results

Approximately 50% of the cows conceived at the first estrus after FSH treatment (Table 1). As determined by ultrasonography, 52 cows were gestating a single fetus. Twenty-nine cows were gestating twins, 17 triplets, and 2 quadruplets. The uterus of 38 additional cows contained only degenerate fetuses, generally three or more degenerate fetuses.

As anticipated, progesterone (P_4) concentrations in the maternal circulation (Fig. 1) were already elevated when the blood collections were initiated at about 120 days of gestation. Circulating concentrations of P_4 were greater for cows gestating multiple fetuses; P_4 concentrations did not differ between dams gestating three vs four fetuses. Results from earlier studies suggested that the corpus luteum was the predominant source of P_4 production and was responsible for maintenance of pregnancy in the cow through the first two trimesters of pregnancy. Studies by other investigators have suggested that extra-ovarian sources contributed to the maintenance of P_4 production during the last trimester of pregnancy. Thus, P_4 concentrations in the earlier stages of gestation are presumably correlated with the amount of functional luteal tissue contained within the ovaries. Whereas circulating concentrations of P_4 during the last trimester of pregnancy result from P_4 production by extra-ovarian sources (i.e., increased production by the placentomes of the placenta or by the adrenals). Perhaps the significant decline at 182 days was associated with the transition from ovarian to extra-ovarian P_4 production. The shorter gestational lengths for dams gestating three or four fetuses resulted in an earlier decline in P_4 (Fig. 1).

Overall, circulating concentrations of estrone sulfate (E_1SO_4) were significantly lower in cows gestating one fetus than in cows with multiple fetuses (Fig. 2). However, the different rates of increase in magnitude of the E_1SO_4 concentrations with time resulted in significant differences among fetal numbers of one, two and three at 294 days of gestation.

Gestational length (Table 2) was significantly shorter ($P < .05$) for cows producing twins than singles, and shorter for cows producing triplets than twins, whereas dams producing triplets, quadruplets and quintuplets had similar gestational lengths. Data for dams that aborted multiple fetuses prematurely were not included in the analysis.

Both calf birth wt and calf survival for the 24 hr after calving (Table 2) decreased as the number of calves per dam increased. Birth wt of individual calves was reduced about 25% for twins vs singles and triplet vs twin, whereas the reduction was smaller among triplets, quadruplets, and quintuplets. Because gestational length had a significant effect on calf birth wt (i.e., .35 kg per day of gestational length), the data were reanalyzed with gestational length as a covariate. Although the adjustment reduced the magnitude of the range in birth wt, the significant effects of calf number and sex of calf remained.

Calf survival within 24 hr after calving did not differ significantly between single and twin calves. Similar results were obtained in our twinning herd for mature cows which were observed closely during calving. However, survival of twin calves from first parity heifers was reduced significantly in the twinning herd.

A complete placenta was collected from a portion of the cows at calving. The total number of cotyledons per placenta (Table 3) did not differ among placentas collected from cows producing single, twin, triplet, or quadruplet calves. Total dry wt of the cotyledons (Table 3) was similar among placentas from cows producing singles, twins, and triplets, but was increased significantly for placentas from cows producing quadruplets. Total wt of the intercotyledonary membranes increased as the number of calves born increased, with the increases being significant for placentas of triplets and quadruplets. However, the total placental wt (cotyledons + intercotyledonary membranes) was increased for only placentas of the quadruplets.

Because birth wt of individual calves decreased as the number of calves produced increased, there was a significant negative correlation between calf birth wt and total calf wt (Table 4). Likewise, total wt of the placenta remained the same or increased slightly with an increase in number of calves; thus, the negative relationship between calf birth wt and placental weight.

As illustrated in Figs. 1 and 2, maternal P_4 and E_1SO_4 concentrations increased with an increase in fetal numbers; thus, significant positive correlations (Table 4) were found between maternal steroid concentrations and total calf weight. Because of the small increases in placental wt with calf number, the relationships between steroid concentrations and placental wt were low. The shorter gestational length for dams with three or four calves reduced the magnitude of the correlations at the later gestational periods.

As greater than 25% of the bovine females gestated two or more calves to term in the present study, it is suggested that ovulation rate is the first limiting factor to increasing beef production. In a subsequent experiment, cows were treated with FSH and slaughtered at 50 days of gestation. Again, the maximum number of viable fetuses was three fetuses per uterine horn or five per uterus. However, because of the increased incidence of abortions for cows gestating triplets and quadruplets, it appears that two fetuses is the optimal fetal number for cattle.

Increasing the number of fetuses per dam resulted in a significant reduction in birth wt and placental wt per calf born and in gestational length for their dams. However, production gains can be obtained from increasing the frequency of twin ovulations and births. Based on the present results, an increase in ovulation rate from one to two resulted in a 1.36-fold increase in number of live calves born, a 1.08-fold increase in total birth wt, and a projected 1.50-fold increase in total calf weaned weight.

Table 1—Induction of multiple births in cattle with FSH

Lactational status	No. cows	Total conception	Type of pregnancy[a]				
			Single	Twin	Triplet	Quad-ruplet	Fetal degeneration[b]
Lactating	189	94(49.7%)	35(18.5%)	18(9.5%)	11(5.8%)	1(.5%)	29(15.3%)
Nonlactating	94	44(46.8%)	17(18.1%)	11(11.7%)	6(6.4%)	1(1.1%)	9(9.6%)
Overall	283	138(48.8%)	52(18.4%)	29(10.2%)	17(6.0%)	2(.7%)	38(13.4%)

[a] Fetal status was evaluated at 45- to 60-day postmating by ultrasonography.
[b] Uterus contained no viable fetuses and a predominance of 3 or more degenerate fetuses.

Table 2—Effect of birth numbers on gestational length and calf birth wt and survival rate[e]

Type of birth	No. cows	Gestational length, day	No. calves	Birth wt, kg	Survival rate[b]
Single	47	291.8[d]	47	44.1[d]	.98[d]
Twin	22	284.3[e]	44	34.8[e]	.93[d]
Triplet	9	273.9[f]	26[c]	25.0[f h]	.74[e h]
Quadruplet	7	272.9[f]	27[c]	21.8[i]	.64[i]
Quintuplet	1	274.0[f]	5	16.6[g j]	1.00[d]

[e] Least squares means. Data for cows aborting twin (n = 1), triplet (n = 5), or quadruplet (n = 1) fetuses between 165 and 244 days of gestation were excluded from the analysis.
[b] Calf survival at 24 hr after calving.
[c] Birth wt of a mummified fetus was excluded.
[d e f g] Means within a column without a common superscript differ (P < .01).
[h i j] Means within a column without a common superscript differ (P < .05).

Table 3—Effect of birth numbers on placental traits at calving[a]

Variable	No. calves born[b]			
	One	Two	Three	Four
No. cows	17	11	6	2
No. cotyledons	70.2	66.1	57.5	88.5
Cotyledonary wt, g				
Wet	1440.4[c]	1372.2[c]	1069.0[e]	2330.8d[f]
Dry	153.6[c]	148.1[c]	125.3[e]	269.5d[f]
Membranal wt, g				
Wet	1510.4[ce]	1755.6[c]	2091.2[d]	2828.5d[f]
Dry	156.6[eg]	184.1[g]	243.0[cf]	353.6d[fh]
Total placental wt, g				
Wet	2950.8[e]	3127.7[c]	3160.1[c]	5159.3[df]
Dry	310.2[g]	332.3[e]	368.3[e]	623.1[fh]

[a] A complete placental unit was obtained from only a portion of the cows calving.
[b] Number of calves born to a cow at the observed calving.
[c d] Means within a row differ among birth groups ($P < .05$).
[e f] Means within a row differ among birth groups ($P < .01$).
[g h] Means within a row differ among birth groups ($P < .001$).

Table 4-Correlations among calf birth wt, placental size, and maternal hormonal concentrations

Variable	Calf birth wt	Total calf wt	Total cotyledonary wt (dry)	Total membranal wt (dry)	Total placental wt (dry)[b]
Calf birth wt	—	-.43**	-.07	-.44**	-.29*
Total calf wt[a]	-.43**	—	.00	.34*	.21
Cotyledonary					
No.	.11	-.12	.69**	.40**	.57**
Wet wt	.03	-.05	.96**	.66**	.86**
Dry wt	-.07	.00	—	.74	.93**
Membranal wt					
Wet	-.36*	.21	.75**	.94**	.90**
Dry	-.44**	.34*	.74**	—	.94**
Total placental wt[b]					
Wet	-.21	.10	.91**	.86**	.95**
Dry	-.29*	.21	.93**	.94**	—
Progesterone					
P-1[c]	-.46**	.23*	.03	.29*	.16
P-2	-.43**	.24*	.03	.31*	.17
P-3	-.52**	.35**	.08	.33*	.26
P-4	-.50**	.32**	.08	.38**	.23
P-5	-.44**	.25*	-.11	.13	-.02
P-6	-.10	.03	-.05	.10	-.02
P-7	-.10	-.24	-.33	-.40	-.44
Estrone Sulfate					
P-1	-.42**	.54**	.09	.30*	.21
P-2	-.52**	.63**	-.06	.31*	.12
P-3	-.53**	.66**	-.13	.30*	.07
P-4	-.47**	.65**	-.05	.17	.04
P-5	-.47**	.54**	.08	.29	.18
P-6	-.54**	.63**	.01	.21	.12
P-7	-.21	.52**	-.69*	.01	-.54

[a] Total wt of calves born per dam.
[b] Total wt of cotyledons plus intercotyledonary membranes.
[c] Gestational period.
*$P < .05$.
**$P < .01$.

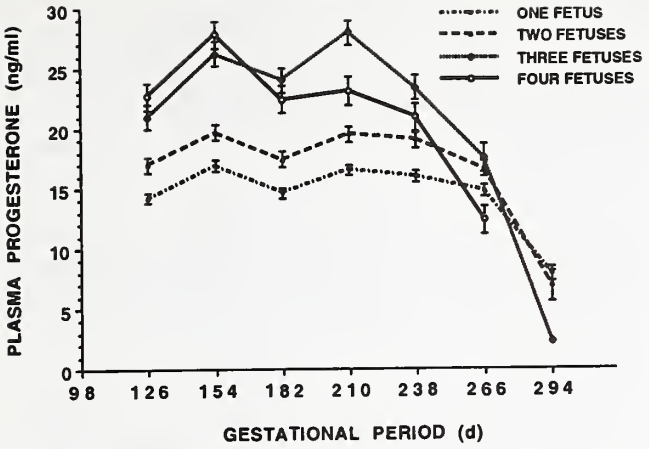

Figure 1 – Relationship between number of fetuses *in utero* and maternal circulating concentrations of progesterone. Progesterone concentrations were proportional ($P < .01$) to number of fetuses *in utero* from 126 to 266 days of gestation, but did not differ for three vs four fetuses ($P > .05$).

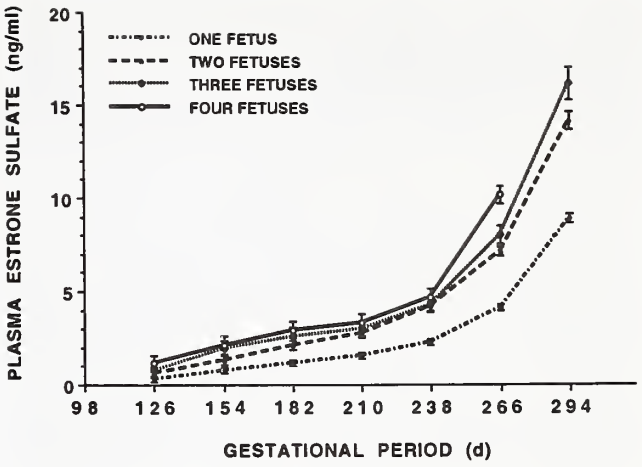

Figure 2 – Relationship between number of fetuses *in utero* and maternal circulating concentrations of estrone sulfate. Estrone sulfate concentrations were lower ($P < .01$) throughout gestation in cows with single vs multiple fetuses. At 266 days of gestation, estrone sulfate concentrations were proportional ($P < .01$) to number of fetuses.

Bovine Embryos from Bluetongue Infected Donors did not Transmit Virus to Susceptible Recipients

John A. Acree, Sherrill E. Echternkamp, Steve M. Kappes, Gary S. Ross, Albert J. Luedke, and James E. Pearson[1]

Introduction

The recent development of methodology for the successful cryopreservation (i.e., ultra low temperature freezing) of bovine embryos has expanded the opportunities for international movement of bovine embryos and has facilitated the exchange of germplasm within and among countries. Unfortunately, frozen embryos also provide an excellent vehicle for the distribution of pathogenic agents (e.g., bluetongue virus, foot and mouth disease, etc.) between livestock populations and between countries. Most pathogens that are on the surface of an embryo with an intact zona pellucida (a translucent mucopolysaccharide shell surrounding the embryo) can be removed by washing. The washing procedure consists of subjecting the embryos to 10 to 12 changes of medium, each change of medium resulting in a 100-fold dilution of the medium. However, some viruses (e.g., infectious bovine rhinotracheitis virus and vesicular stomatitis virus) adhere firmly to the zona pellucida and are not removed by the 10 to 12 changes of medium. The objective of this study was to determine the risk of transmitting the bluetongue virus by washed bovine embryos from infected cows. The present study was conducted with a sufficient number of animals to estimate the probability of bluetongue virus transmission by the transfer of embryos from an infected donor, to mimic as closely as possible the natural infection of embryo donors, and to collect the embryos at various periods during the donors' interaction with the virus.

Procedure

Healthy, nonpregnant, nonlactating, BTV-susceptible, bovine heifers (n=59) were infected with BTV serotype 11 strain C075B300 (BTV-11) by bites from infected *Culicoides variipennis* ssp. *sonorensis*. The midges (gnats), in lots of 25 to 100, were given access through a nylon membrane to a shaved area on the back of the donor heifers for 30 min. In addition, the heifers were inoculated intradermally with a suspension of homogenized infected *C. variipennis sonorensis* at the end of the midge exposure. Virus isolation and antibody titers confirmed that all 59 heifers became infected with BTV-11. *C. variipennis* is a biting midge and the principal vector of BTV in North America. The midges used in this study came from the AK colony at the Arthropod-borne Animal Disease Research Laboratory at Laramie, Wyoming.

Between 5 and 8 days after infection, the 59 viremic donor heifers were given twice daily injections of FSH for 4 days (total dosage of 34 mg) to initiate superovulation and 35 mg of prostaglandin $F_{2\alpha}$ on the last day of FSH treatment to induce estrus. Heifers were artificially inseminated at 12, 24, and 36 hr after onset of estrus (day 0) with semen from bulls that were negative for BTV. Embryos were collected nonsurgically on day 7 or 8. Isolation of bluetongue virus and elevated antibody titers in blood samples indicated that all 59 donors were viremic at embryo collection (i.e., "acute" donors). At 1.5 and 3 mo after infection, a second and third collection of embryos from these donors were attempted. Blood taken at the time of embryo collection contained antibodies to BTV-11 but no viruses were isolated so the animals were considered to be "convalescent" donors.

At all three embryo collections, the embryos were washed 10 times in 2 ml aliquots of fresh sterile medium. Embryos were agitated throughout the wash volume, and a new pipet was used to carry them to the next wash in 20 µl of fluid. All embryos (up to 10) from a single donor were washed together. Washed embryos were examined microscopically at 50X magnification. Only grade 1 and 2 morulae and blastocysts with an apparently intact zona pellucida were selected for transfer into BTV negative recipient females. Each selected embryo was identified to its dam and sire and was transferred nonsurgically into a recipient either within a few hr after collection or frozen for later transfer. Embryos unacceptable for transfer were frozen separately for each donor, and virus isolation was attempted later at the National Veterinary Services Laboratory, Ames, Iowa.

After receiving embryos, the recipients were observed for signs of disease and periodically tested for BTV group specific antibody. If the recipient did not become pregnant to the embryo transfer but remained free of signs of, or antibody to, BTV for at least 60 days after receiving the embryo, it was assumed that there had been no lateral transmission of virus from infected donor to recipient via the embryo. If the recipient became pregnant but remained free of signs of, or antibody to, BTV for at least 60 days after abortion or parturition, it was assumed that there had been no transplacental exposure to a viremic fetus that had been infected vertically via the embryo. If the offspring remained free of signs of, or antibody to, BTV for at least 60 days after its birth, it was assumed that there had been no vertical transmission of virus from infected donor to offspring via the embryo. Pregnant recipients were housed at the U.S. Dairy Forage Research Center, Prairie du Sac, Wisconsin, (i.e., a BTV-free area) from May 4 to October 28, the vector season for *C. variipennis* in Nebraska.

Results

A total of 169 embryos were collected from 34 of 59 viremic donors (Table 1). Virus was not isolated from the 57 nontransferable embryos examined. Recipients of 108 fresh and 2 thawed transferable embryos remained free of evidence of BTV infection for more than 60 days after embryo transfer. The 36 calves (Table 1) resulting from these embryo transfers had no evidence of BTV infection at 60+ days of age and their surrogate dams were also free of signs of BTV infection at that time; two embryos were not used.

A total of 141 embryos (Table 1) were collected from 44 convalescent donors (i.e., second and third embryo collection at 1.5 to 3 mo, respectively, after BTV infection). Virus was not isolated from blood samples taken from the donors at the time of embryo collection or from 20 washed embryos submitted for virus isolation. Recipients of 59 fresh and 62 thawed embryos remained free of evidence of BTV infection

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for more than 60 days after embryo transfer. The 52 calves (Table 1) resulting from these transfers had no evidence of BTV infection at 60+ days of age; their surrogate dams also remained free of BTV infection. Furthermore, no BTV virus was isolated from the flush fluids or wash fluids. Failure to isolate BTV virus from flush fluids of acute or convalescent donors may have been because very few of the embryo collections contained appreciable amounts of blood. In summary, we found no evidence of BTV-11 transmission from viremic or convalescent donors to susceptible recipients or their offspring by 7- to 8-day embryos that had been washed according to the recommendations of the International Embryo Transfer Society.

Table 1—Incidence of bluetongue virus (BTV) transmission

Type of donor	Donors	Embryos		Recipient		Calves
		Transferable	Nontransferable	Pregnant	Nonpregnant	
Acute:						
Total no.	59 (34[a])	112	57	36	74	36
No. with BTV	59	0	0	0	0	0
No. seropositive	59	—	—	0	0	0
Convalescent:						
Total no.	59 (44[a])	121	20	52	69	52
No. with BTV	0	0	0	0	0	0
No. seropositive	59	—	—	0	0	0

[a] Number of donors contributing embryos are in parentheses.

Superovulation of Cows by Initiating FSH Treatments During the First Few Days After Estrus

Andrew J. Roberts and Sherrill E. Echternkamp[1]

Introduction

Superovulation of cattle is a technique being used in conjunction with embryo transfer to expedite the propagation of animals with genetic merit for desirable traits. Briefly, these techniques involve the induction of multiple ovulations during one estrous cycle by administering multiple injections of a hormone called follicle stimulating hormone (FSH). Under ideal conditions this treatment will result in multiple embryos. These embryos can then be collected from the cow and transferred to recipient cows of lesser value, which act as surrogate dams. These techniques allow the production of many calves from one cow within a year as opposed to the normal rate of one calf per cow per year.

One major problem often associated with superovulation of cows has been the large variation in the number of ovulations and(or) embryos that result from this treatment. In standard superovulation protocols, injections of FSH are usually initiated during the middle of the estrous cycle (9 to 12 days after estrus). Recent research provides evidence that the large variation in superovulation response may be due in part to the variation in the development of follicles present on the ovaries when treatment with FSH is initiated. Some researchers speculate that the presence of a large follicle on the ovaries of a cow may suppress the ability of FSH to induce the development of other follicles, thereby decreasing the number of follicles that mature and ovulate in response to FSH. One possible approach to overcome this problem would be to begin FSH treatments during the first few days after estrus when the population of follicles on the ovaries is less variable and consists mainly of small follicles. Therefore, it was the objectives of the following experiments to 1) determine an appropriate dosage of FSH to use when superovulating cows during the early stage of the estrous cycle and 2) evaluate whether or not treatment with FSH during the early stage of the estrous cycle (within the first few days after estrus) would result in better superovulation responses that were less variable than standard superovulation protocols that initiate treatment with FSH during the middle of the cycle.

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Procedures and Results

The objective of Experiment 1 was to compare the superovulation response in cows that were treated with different dosages of FSH (Schering-Plough Animal Health) beginning one to four days after estrus. Results from this study would then be used to compare superovulation responses in cows treated early in the cycle to cows treated in the middle of the cycle (Experiment 2). Injections of FSH were given twice daily (morning and evening) for five days. The amount of FSH given in each injection on each of the five days of treatment was 5, 5, 4, 3, and 3 mg for a total of 40 mg over the 5-day period for cows in the Low dosage treatment group and was 7, 6, 5, 4, and 3 mg for a total of 50 mg over the 5-day period for cows in the High dosage group. An injection of prostaglandin (Lutalyse, Upjohn Co.) was given at the last FSH injection and again the next morning to bring cows into heat. All cows were then bred by bulls and slaughtered six to eight days later. The reproductive tracts were collected and flushed to recover embryos. During the second or third day of FSH treatments, three cows from the Low group were mistakenly injected with doses of FSH scheduled for the High group. Therefore these three cows received a higher dose than scheduled. Results from these three cows were analyzed separately and are designated as the Mix group. Results from this experiment are summarized in Table 1.

Table 1—Summary of ovarian response and embryo production in cows treated with different levels of FSH during the early stage of the estrous cycle

	FSH Dose		
	Low	High	Mix
Number cows treated	9	12	3
Number cows with >2 ovulations	5	7	3
Number of ovulations[a]	19 ± 3.7	25 ± 9.2	44 ± 2.8
Range in ovulations[a]	15–34	4–34	38–47
Number unfertilized ova[a]	7.6 ± 4.6	5.8 ± 2.6	2.3 ± 1.9
Number transferable embryos[a]	6.6 ± 2.4	4.4 ± 2.7	25.6 ± 5.2[b]
Total number of ova and embryos[a]	16.6 ± 2.4	11.6 ± 2.8	34 ± 5.1[b]

[a] Values represent the mean ± SE or range from animals with more than two ovulations.
[b] Values are statistically different from the Low and High dose.

The recovery rates of embryos and ova (i.e., the number of ova and embryos recovered divided by the number of ovulations for each cow) were 87, 64, and 78% for the Low, High and Mix groups, respectively. No differences for any of the parameters measured were observed between the Low and High dosages. However, more transferable embryos were obtained from the Mix group than from either the Low or High treatment groups. In addition, there was a very consistent superovulation response in the Mix group. These results provide preliminary evidence that superovulation may be best achieved by administering FSH in such a way that the dosage of FSH increases over the first few days and then declines, as opposed to the standard superovulation protocol that utilizes a declining dosage of FSH overtime.

All the treatments used in this experiment resulted in respectable ovulation rates. However, the number of transferable embryos or embryos that would have been suitable for transfer to recipient cows was much lower than the ovulation rates achieved, especially in the Low and High groups. Because the total number of embryos and ova recovered were generally high incomparison to the number of transferable embryos, conditions in this study may not have been favorable for optimal embryo development. Although no definite causes have been determined at this time, it is speculated that changes in circulating levels of estrogen resulting from the superovulation treatment may have contributed to the low proportion of transferable embryos obtained. Superovulation treatments increase the levels of circulating estrogen, because estrogen is produced primarily by large follicles and the number of large follicles is increased by FSH. Normally when cows are superovulated during the midcycle (9 to 12 days after estrus), any negative effects that sustained high levels of estrogen may have on the reproductive tract prior to breeding would probably be prevented by the high circulating levels of progesterone that occur during this stage of the cycle. However, during the early stages of the cycle, progesterone levels are very low and therefore high levels of estrogen may adversely affect the reproductive tract during this time, thereby interfering with subsequent development of embryos. Support for this speculation is provided by the fact that five cows from the Low group and seven cows from the High group were observed in heat after the second or third day of FSH treatment indicating that estrogen levels were elevated. In standard superovulation treatments administered in the middle of the cycle, cows do not generally show signs of heat. One possible method to overcome the adverse effects of sustained high levels of estrogen during the early stage of the cycle would be to give progesterone to cows during the FSH treatment.

Results from this experiment provided preliminary evidence that superovulation *may* be best achieved by administering increasing doses of FSH during the first few days and then decreasing the dose over the remainder of the treatment. In addition, it was speculated that treatment with progesterone or a progesterone-like substance *may* increase the number of transferable embryos by overriding the negative effects that prolonged high levels of estrogen may have on the reproductive tract in the absence of high progesterone. Therefore, these conditions were used in the following experiment to determine if greater numbers of transferable embryos could be obtained.

Experiment 2 compared the superovulation responses in 19 cows that were treated with FSH beginning on 1 or 2 days after estrus (designated as Early cycle group) to 25 cows treated with FSH beginning on day 10 or 11 after estrus (designated as Midcycle group). Cows used in this study were stratified by breed (Braunvieh, Gelbvieh, Simmental, Pinzgauer, MARC I, MARC II, MARC III, and crossbred cows from the twinning herd) and by age (3 to 10 years) within the two groups. The dosage of FSH given during each injection on each of the 5 days of treatment were 5, 6, 4, 3 and 3 mg for a total of 42 mg over the 5 day period. In addition, cows in the Early cycle group were implanted with a Norgestomet implant (Syncro-Mate-B, Sanofi Animal Health) on the first day of FSH treatment. Because Norgestomet is a progesterone-like substance, it was expected that the implant may reduce the negative effects that high levels of estrogen during FSH treatment may have on the reproductive tract in the early stage of the cycle when progesterone levels are low.

On the day before FSH injections were initiated the ovaries of each cow were visualized by ultrasonography to determine whether the size of follicles present on the ovaries of cows in the Early and Midcycle groups were different. An injection of 1 mg fenprostalene (Bovilene, Syntex) was given at the last FSH injection and again the next morning to bring cows into heat. Implants were removed from the Early group at the time of the second Bovilene injection. Cows were artificially inseminated at 12 and 24 hours after first observed in standing heat. Cows that were not seen in heat were artificially inseminated at 72 and 84 hours after the first fenprostalene injection. All cows were slaughtered 6 to 8 days after breeding and their reproductive tracts were flushed to recover embryos. The number of cows observed in heat and ovulating in this experiment are summarized in Table 2.

Table 2—Response of cows to treatment with FSH in the Early and Midcycle of the estrous cycle

Treatment group	Number of treated cows	Number in heat	Number ovulating	Number with >2 ovulations
Early cycle	19	7	9	6
Midcycle	25	12	9	5

No differences between the two groups were observed for any of the above parameters. The number of cows exhibiting estrus and ovulating was unusually low. The reasons for this are not clear. However, in the previous experiments, Lutalyse was used instead of Bovilene.

To determine if the superovulatory response was different between the two groups, the data for those cows which had more than two ovulations were evaluated. Table 3 summarizes ovulation rates and the number of ova and embryos obtained from the two treatments.

Table 3—Ovulation response and embryo production in cows treated with FSH in the Early and Midcycle of the estrous cycle

	Treatment group	
	Early cycle	Midcycle
Number of cows with >2 ovulations	6	5
Number of ovulations[a]	26 ± 6.28	49.6 ± 25.8
Range in number of ovulations[a]	15–55	12–150
Number unfertilized ova[a]	2± .44	5.8 ± 4.57
Number transferable embryos[a]	8.0± 3.59	5.4 ± 1.54
Total number ova and embryos[a]	13.33 ± 3.2	14.4 ± 3.36

[a] Values represent the mean ± SE or range from animals with more than two ovulations.

The recovery rate of ova and embryos obtained per ovulation was 59% and 51% for the Early and Midcycle groups, respectively. Treatment of cows in the Early cycle group with the Norgestomet implant appeared to prevent cows from showing heat during the FSH treatment and the proportion of transferable embryos collected per ovulation or per total number of embryos in this group was not different from those obtained in cows treated in Midcycle. However, the proportion of transferable embryos collected in the Early cycle was very similar to the Mix group in Experiment 1. Therefore, it is questionable whether the implant had any beneficial effect on embryo development. In addition, the consistency of the superovulation response observed in the Mix groups of Experiment 1 was not observed in this experiment. Therefore, the fashion in which FSH was administered (i.e., increasing dosages followed by decreasing dosages) may not be advantageous over the standard declining dosage procedure. Although the average number of transferable embryos was numerically higher in the cows treated with FSH early in the cycle, no statistical differences were observed for any of the parameters evaluated, except that the largest follicles on the ovaries of cows before FSH treatment were smaller in the Early cycle group than in the Midcycle group.

Results from these experiments *do not* demonstrate any advantage of superovulating cows during the early stage of the cycle. However, the small number of animals that had multiple ovulations in Experiment 2 prohibits any firm conclusions from this study. Future studies will focus on evaluating the superovulation response in cows treated in a similar fashion as in Experiment 2, except that cows will be injected with Lutalyse after the FSH treatment instead of Bovilene to try to increase the number of cows that exhibit estrus, thereby allowing a more thorough examination of these superovulation procedures.

Factors Involved in Regulating the Development of Ovarian Follicles in Cattle

Andrew J. Roberts, Sherrill E. Echternkamp, Judith M. Grizzle, and Thomas H. Wise[1]

Introduction

The inability to regulate ovarian follicular development is a major obstacle to improving reproductive efficiency in cattle whether the objective be estrous cycle regulation, multiple ovulations and births, superovulation for embryo transfer, a shorter postpartum anestrous period or younger age at puberty. Similarly, normal growth and selection of ovulatory follicles are required for sexual behavior, for maturation and release of viable oocytes and for preparation of the uterine environment for gamete transport, fertilization and embryonic development. Increasing reproductive efficiency is critical to increasing livestock production as reproductive losses in cattle range from 20 to 35%. Research outlined in this report is focused on providing insight into how circulating hormones (i.e., the endocrine system) and factors produced within the ovary (i.e., paracrine and autocrine systems) are involved in regulating follicular development and ovulation rate.

It is well documented that hormones secreted from the pituitary (i.e., LH and FSH) stimulate follicle development. However, it now appears that factors produced within the ovaries may also be important for regulating follicular development. Recently, the following peptides and proteins have been shown to exert either stimulatory or inhibitory effects on ovarian follicular growth, maturation and steroidogenesis: insulin-like growth factors (IGF-I and -II), transforming growth factors (TGF) alpha and beta, epidermal growth factor, platelet-derived growth factor, fibroblast growth factor, plasminogen activator, interleukin-I, activin, and inhibin. Although this list contains several factors, it is by no means complete. In fact, many new factors are identified each year that appear to have roles in regulating follicular development. Obviously, the mechanisms involved in controlling follicular development are probably very complex and depend on the actions and interactions of both circulating hormones and substances produced in the ovary. Research is currently being conducted to determine the role that IGF-I, TGF-alpha, TGF-beta, inhibin, and interleukin-I have in regulating follicle development and ovulation rate.

Research and Procedures

Because the factors of interest are produced and act within the ovary, it is very difficult to determine their actions by simply evaluating blood samples from live animals. Therefore, most of the studies conducted to date have involved the collection of follicles from ovaries obtained from cows slaughtered at different stages of the estrous cycle. The follicular fluid and cells from these follicles are then used to measure the amount of the different factors of interest. In addition, cells from follicles are also cultured in incubators for several days to determine the effects that treatment with the various growth factors have on the cells' ability to proliferate and perform the necessary biochemical processes needed for follicular development (i.e., the production of androgens, estrogens, and progesterone).

Interest in the role that IGF-I has in regulating follicular development stems from research findings that IGF-I enhances the response of follicle cells to FSH and findings that IGF-I levels are higher in the blood and follicular fluid of twin-producing cows. However, the role that IGF-I has in regulating follicular development is now further complicated by the fact that several different proteins, called IGF-binding proteins, are also found in developing follicles. These proteins bind to IGF-I and either prevent or enhance the actions of IGF-I. Research is now underway to characterize levels of IGF-I and of the various IGF-I binding proteins in follicles at different stages of development. Preliminary results indicate that the levels of IGF-I may not differ very much between follicles at different stages of development. However, the quantity and types of IGF-I binding proteins decrease with follicular development. It is speculated that changes in IGF-binding protein profiles may be important in regulating the maturation of small follicles to large follicles.

Research interest in the roles that TGF-alpha and TGF-beta have in regulating follicular development stems from the discovery that these two proteins are produced by cells within the follicle. To date, research has shown that these two factors may be important in regulating the growth of follicles. Treatment of cells with TGF-alpha stimulates cells to divide (i.e., proliferate) during culture whereas treatment with TGF-beta prevents proliferation in cells treated with TGF-alpha. These two growth factors may therefore provide the mechanism by which growth of follicles can be controlled, since follicle growth is in part due to an increase in cell number within a follicle. Research is now underway to determine if levels of TGF-alpha are higher in healthy, developing follicles and if levels of TGF-beta increase in follicles that regress or fail to develop to a large ovulatory size. In addition, research is underway to determine what regulates the production of these growth factors. It is possible that in the future the amounts of these factors being produced within follicles may be manipulated to enhance the reproductive efficiency of cattle.

It has been known for many years that inhibin is secreted from follicles and acts at the level of the pituitary to suppress the release of FSH. However, little research has been conducted to determine what direct effects inhibin may initiate on follicular development. Recent research from other laboratories proposed that interactions between different cell types in the follicle may play a role in regulating the number of ovulations. Specifically, interactions of inhibins and steroids were different in humans or primates that normally have only one ovulation and species of animals that have more than one ovulation. Since treatment with FSH is commonly used to induce multiple ovulations in cattle, it was expected that if interactions of inhibin and steroids existed in follicles of cows, the differences in these factors would be observed in cows receiving FSH and cows not receiving FSH. Results from a study designed to test this hypothesis demonstrate that production of inhibin and androgen was higher in follicles from FSH-treated cows than from nontreated cows. Conversely, estrogen was lower in FSH- treated cows. Since inhibin is produced by one cell type (i.e., granulosa cells) and androgen is produced by another cell type (i.e., theca cells) in the follicle, these results provide preliminary evidence that inhibin and steroid interactions may also exist between different cell types in follicles of cows. Future studies will focus on fur-

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ther understanding the roles that inhibin and steroids have in regulating ovulation rate.

The last area of research pertaining to how secretory factors produced within follicles may regulate follicular development involves interactions of cells from the immune system with cells in follicles. For many years the process of ovulation has been said to have many similarities to inflammatory responses that occur in other tissues. Recently, a study was conducted to determine if macrophages, which are cells from the immune system, migrate into follicles in a similar fashion as would occur in response to an injury. Results from this study demonstrate that the number of macrophages found inside large follicles does increase during the two to three days prior to ovulation. These results provide information that macrophages may also play an important part in regulating follicular development and ovulation. Since macrophages can secrete several factors that can alter the function of many different types of cells, it was of interest to examine the effects that some of these factors may have on follicular development. One factor secreted by macrophages, interleukin-I, has been shown by other laboratories to alter the secretory function of granulosa cells. To further examine how interleukin-I may alter follicular development, a study was designed to evaluate theca cell function after treatment with interleukin-I. Results from this study indicated that interleukin-I can change the amount of androgen and progesterone that is secreted by theca cells. The exact roles of interleukin-I and macrophages in regulating follicular development and ovulation are unknown, but these results do indicate that they may be involved in the control of follicular development and secretion.

Results from the studies discussed above provide additional support for the concept that substances produced within follicles may play important roles in regulating follicular development and ovulation. However, our knowledge of what occurs during the development of follicles is still very limited. The process of follicular development is very wasteful in cattle because less than one percent of the follicles that begin to develop ever reach the stage of ovulation. Although application of the research described above may not readily be apparent, it is expected that by gaining a better understanding of all the processes involved in follicular development, we as scientists will more likely be able to develop procedures to enhance the reproductive efficiency of cattle.

The Relationship of Metabolic Hormones, Nutrition, and Postpartum Anestrus in Different Biological Types of Cattle

Andrew J. Roberts, Russell A. Nugent III, Thomas G. Jenkins, and John M. Klindt[1]

Introduction

Restricted energy intake can suppress the overall productivity of cattle. Restricted energy intake can decrease overall productivity of beef cows through, among other traits, decreased milk production, calf performance, and reproduction. However, failure of a cow to conceive is the major component affecting the overall production efficiency of the cow herd. In a companion report included in this publication ("Postpartum Interval Is Influenced by Nutritional Environment and Biological Type"), we demonstrated that the postpartum intervals for breeds of cattle with different genetic potentials for growth and milk production were differentially affected by restricted energy availability. This report, along with numerous other studies, confirms the fact that limited availability of energy can increase the time it takes cows to resume estrous cycle activity after calving. However, the mechanism(s) by which limited energy intake influences resumption of estrous activity after calving is not known. The objective of this study was to identify how changes in metabolic hormones correspond to the time from calving to resumption of cyclicity. In addition, results from this study may explain why diverse biological types of cattle respond differently to restricted energy availability.

Procedure

Cattle used in this study were purebred Angus, Braunvieh, Charolais, Gelbvieh, Hereford, Limousin, Pinzgauer, Red Poll, and Simmental. Four cows from each breed were fed daily rations of ground alfalfa hay and shelled corn containing 130, 170, 210, and 250 kcal of metabolizable energy x body weight$^{-.75}$ (i.e., metabolic weight) for four years. Rations were increased by 25% after calving to account for the increased demand of lactation. Additional information on the treatment, feeding and handling of these cows is included in the companion report entitled "Postpartum Interval Is Influenced by Nutritional Environment and Biological Type" found elsewhere in this publication.

In 1991, 121 of 144 cows calved. Beginning at approximately 3 weeks after calving, cows were bled once per week for at least 15 weeks. In addition, a subset of cows that included the Red Poll, Hereford, Charolais, and Braunvieh cows on the 2 lowest energy levels (i.e., 130 and 170 kcal ME/wt$^{-.75}$/day) were bled every 15 minutes for 6 hours on weeks 2, 3, 4, 6, 8, and 10 after calving. These intensive blood-sampling periods were conducted because levels of some hormones fluctuate in pulsatile fashions, thereby making it difficult to draw conclusions from a single sample at any one time. Serum from all of the blood samples was frozen for future analysis of several metabolic hormones.

Results

To date, two hormones have been analyzed, growth hormone (GH) and insulin-like growth factor-I (IGF-I). Results indicate that maintenance of cattle on the lower levels of energy intake suppresses levels of IGF-I. In contrast, GH appears to be increased in cows maintained on low levels of energy. Thus, chronic (i.e., 4 years) restriction of energy intake affects IGF-I and GH in opposite fashions. It was of interest to determine whether the levels of these hormones would be useful indicators of when cows would resume cycling. The fact that GH is secreted in a pulsatile fashion makes this hormone less desirable as a possible indicator of reproductive status because several blood samples would be required. Levels of IGF-I, however, were relatively consistent from one week to another. Therefore, IGF-I concentrations in serum at three weeks after calving were evaluated as an indicator of length of time it took cows to resume cycling. Analysis indicated that postpartum interval was negatively associated with IGF-I for cows maintained on the two lowest levels of energy. In simplistic terms, these results indicate that when energy intake is low, cows with high levels of IGF-I will likely resume cycling sooner than most of the cows with low levels of IGF-I. However, some cows with low levels of IGF-I did resume cycling at similar times as cows with high levels of IGF-I. These results indicate that low levels of IGF-I are not as consistent for predicting postpartum intervals as high levels are. Because of this observation, measuring circulating IGF-I levels will probably not be acceptable as a management tool for predicting length of postpartum interval.

Ongoing research will focus on evaluating other metabolic factors, including insulin, glucose, and thyroid hormones. Our future objective is to determine whether these factors may be involved in mediating nutritional effects on reproduction. In addition, research is underway to determine if low levels of IGF-I may be perceived differently by tissues involved in regulating ovarian function (i.e., the hypothalamic-pituitary-ovarian axis), thereby resulting in the wide range of differences in length of postpartum interval observed in cows with low IGF-I levels.

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Scrotal Thermography as a Tool for Predicting Semen Quality and Natural-Mating Fertility in Young Beef Bulls

Donald D. Lunstra and Glenn H. Coulter[1]

Introduction

The scientific literature offers only sparse information on mechanisms controlling reproductive function in beef bulls and indicates that there are very few consistent relationships between the commonly evaluated male reproductive characteristics and variations in natural-mating fertility of beef bulls. Current techniques for evaluating and predicting reproductive potential of bulls are ineffective, and the beef cattle industry selects breeding males on the basis of appearance, growth rate, size, and other factors of little relationship to reproductive potential. Reproductive merit is five times more important economically than is growth performance and at least ten times more important than product quality for the average cow-calf producer. However, little selection pressure for fertility has been applied to beef sires (bulls) in North America because of the relative inaccuracy of methods available for evaluation and prediction of breeding potential and fertility in beef bulls. For example, detailed semen evaluation in groups of yearling (less than 18 mo of age), physically-sound beef bulls rarely eliminates more than 5% as potential breeding sires, yet single-sire mating using bulls from the 95% that passed semen evaluation still results in dramatic variation in sire fertility. Many producers obtain little or no information on the reproductive status of their bulls prior to use in natural-mating programs, and this is particularly true for the use of young breeding bulls (i.e., yearlings) in the beef cattle industry. The lack of effective means for selecting males with superior fertility is due primarily to two factors: 1) the lack of information on basic measurable characteristics of male reproduction that are related to sire fertility, and 2) the cost and difficulty of obtaining accurate fertility data on individual sires.

Development of valid knowledge of the limiting mechanisms in male reproduction and establishment of effective, reliable techniques for evaluating the characteristics of male reproduction that are related to fertility are prerequisite to improving the productivity of the livestock industry. It is known that thermoregulation in the testes is essential for sperm production. For normal spermatogenesis in the bull, the testes in the scrotum must be maintained at a temperature approximately 5° to 8°F lower than normal body temperature (101°F). Adverse effects of elevated testicular temperature on sperm production, semen quality, and male fertility have been documented for many species of domestic animals. Recently, other researchers have shown that the surface temperature of the scrotum is highly correlated with deep testicular temperature, and that infrared thermograms (images of radiated heat emission) of the scrotal surface provide accurate information about testicular thermoregulation in domestic species. However, the relationships between scrotal thermography (infrared thermography of the scrotal surface) and various aspects of semen quality and fertility remain unknown in the beef bull. The objectives of the following study were to evaluate the potential usefulness of scrotal thermography as a tool for predicting the natural-mating fertility of yearling beef bulls, and to obtain standard breeding soundness information for comparison to scrotal thermography and bull fertility data.

Procedures

Infrared thermography of the scrotal surface was performed in late April on 73 yearling beef bulls (14 mo of age), representing nine pure breeds and three composite breedtypes of beef bulls. Scrotal thermograms were obtained with a hand-held infrared video camera (Thermovision 782 System; AGA Infrared Systems AB, Danderyd, Sweden) positioned approximately 3 ft from the rear of each bull and perpendicular to the paired testes in the scrotum, and thermogram data recorded onto videotape for subsequent analyses of computerized (pixelized) thermal images. The average scrotal temperature (AST = average of all thermogram temperature pixels over the scrotum), temperature at the top (STT) and bottom (STB) of the scrotum, scrotal temperature gradient (STG = difference between scrotal surface temperature at top and bottom of scrotum), thermal class (TC = normal, questionable or abnormal, based on evaluation of each bull's scrotal surface thermal pattern and uniformity of temperature gradient), and ambient temperature (AT = air temperature at moment thermogram was taken, average = 50°F) were recorded for each thermogram. Within 2 days after thermography, each bull was subjected to testes measurement [testis length (TL) and scrotal circumference (SCR) were measured], scrotum and testes were palpated for abnormalities, and semen was collected twice (once daily) from each bull via electroejaculation. Paired testis volume (PTV) was calculated using the formula of Lunstra et al., 1988 [PTV = 0.0396 (average TL)$(SCR)^2$]. At semen collection, ejaculate volume, sperm concentration, progressive motility, sperm morphology, and acrosome integrity were assessed for each ejaculate, using the methods described by Lunstra and Echternkamp, 1982. Semen was maintained at 99°F for evaluation of progressive motility determined immediately from duplicate estimates using a microscope (400x magnification). Sperm concentration was determined from spectrophotometer (550 nm) counts of duplicate semen aliquots diluted 1:200 with 1% formalin in 0.9% saline. Live sperm and sperm abnormalities were quantitated microscopically for each ejaculate by scoring 100 sperm in smears stained differentially with eosin-fast green. Acrosome morphology was assessed microscopically for each ejaculate by scoring 100 sperm on coverslipped slides after fixation of semen aliquots by 1:10 dilution with 3% glutaraldehyde in 0.9% saline. Three weeks after semen evaluation, 30 of these 73 bulls were selected for natural mating and exposed single-sire to approximately ~18 heifers per bull (15 mo of age; matched by breedtype) during a 45-day pasture-breeding period. Based on testis size and semen quality, all bulls used for mating had achieved a score of satisfactory on the standard breeding soundness examination. Pregnancy rate in heifers was determined by rectal palpation at 80 day after the end of the pasture-breeding period. All pregnancy rates reported herein are expressed as % pregnant, based on number of heifers pregnant divided by the number of heifers exposed per bull.

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Results & Discussion

Among the 73 bulls evaluated, neither scrotal circumference nor paired testes volume were correlated to any characteristics of the scrotal thermograms (Table 1). Most thermogram characteristics (AST, SST, STB and STG) were not correlated (P>.14) with the various aspects of semen quality, including sperm concentration, % progressive motility, live sperm, sperm morphology, and proximal droplets, among the 73 bulls evaluated (Table 1). However, thermogram classification (TC; where normal = 1, questionable = 2, and abnormal = 3) was correlated significantly with % normal heads (r=-.23, P=.05), % proximal droplets (r=.27, P=.02) and % normal acrosomes (r=-.21, P=.07). Thus, yearling beef bulls exhibiting abnormal thermograms also produced sperm with higher percentages of abnormal heads, abnormal acrosomes, and proximal droplets. These results provide additional confirmation of data indicating that reduced semen quality is related to impaired thermoregulation in the bull testes (Coulter, 1988). More importantly, the pregnancy rate achieved by the 30 bulls used in the breeding trial was strongly correlated with their TC (r=-.46, P=.01, Table 1) and also was correlated significantly with all other scrotal thermogram characteristics (AST, P=.03; STT, P=.07; STB, P=.02; and STG, P=.05; Table 1). Pregnancy rate achieved by bulls exhibiting abnormal thermogram patterns was 15 to 17% lower (P<.01) than the pregnancy rates achieved by bulls with normal and questionable thermogram patterns (Fig. 1).

All 30 bulls used for fertility tests had exhibited acceptable semen quality and had achieved a score of satisfactory on the standard breeding soundness examination (BSE) prior to use in breeding trials. It is not surprising that total BSE score within TC groups did not differ (P=.40) for the 30 bulls used for fertility tests (Table 2). Thus, bulls exhibiting these three different thermogram classes were present among bulls that were acceptable for use as breeding sires, based on the best standard breeding-soundness criteria available. Among the 30 bulls used for fertility tests, the only semen characteristics related to pregnancy rate (Table 1) were % normal sperm heads (r=.64, P<.01) and % proximal droplets (r=-.32, P=.08). Examination of means within TC group (Table 2) for semen characteristics revealed that only very small numerical differences in % normal sperm head morphology (91 to 94%) and % proximal droplets (1.2 to 3.4%) existed among the three TC groups. In fact, statistical analyses indicated that the three TC groups did not differ significantly in any of the seven characteristics of semen quality (Table 2). Although % normal sperm heads and % proximal droplets were significantly correlated with pregnancy rate, the narrow range exhibited by these semen characteristics precluded elimination of potential sires on that basis.

When % normal heads was included in the statistical analysis as a covariate, the effect of TC on pregnancy rate remained important, and differences in pregnancy rates still remained among normal, questionable and abnormal TC bulls (84 ± 3%, 83 ± 3% and 70 ± 3%, respectively; P<.01, Figure 1; compare to pregnancy data in Table 2). Even after adjustment of data for the significant effect of % normal sperm head morphology, the pregnancy rate achieved by bulls exhibiting abnormal thermogram patterns was 13 to 14% lower (P<.01) than the pregnancy rates achieved by bulls with normal and questionable thermogram patterns (Figure 1).

Among the 30 bulls used for fertility tests, the relationships between pregnancy rate and measurements of testis size approached significance (Table 1). Both scrotal circumference and paired testes volume were correlated negatively with pregnancy rate, indicating that larger testes were associated with reduced pregnancy rate. Examination of means within TC group (Table 2) for testis size indicated that bulls exhibiting abnormal thermogram patterns had significantly larger testes than did normal and questionable TC bulls. During testis measurement, palpation of the scrotum, testes and epididymides had revealed no detectable abnormalities among these 30 bulls. Although detected only with thermography, it is possible that abnormal testicular thermoregulation may be associated with a small increase in testis size. It is interesting to note that, in general, scrotal temperatures were significantly different in abnormal TC bulls, compared to normal and questionable TC bulls (Table 2). While temperature at the top of the scrotum did not differ among TC groups (P=.65), temperature at the bottom of the scrotum was higher (P=.003) in abnormal TC bulls than in normal or questionable TC bulls. The higher temperature at the bottom of the scrotum in abnormal TC bulls was reflected in a reduced temperature gradient from top to bottom of the scrotum (STG, P=.003 to P=.0001) and in an increased average scrotal surface temperature (AST, P=.02) in abnormal TC bulls compared to values for normal and questionable TC bulls (Table 2). These data indicate that abnormal TC bulls exhibit a reduced ability to maintain a effective thermoregulation gradient from top to bottom of the testes, and that abnormal TC bulls produce semen of acceptable quality (evaluated with standard techniques) but achieve reduced pregnancy rates when used for natural mating (Fig. 1).

In summary, infrared thermography of the scrotal surface shows promise as a tool for providing data that is easily obtained and appears to be a relatively accurate predictor of impaired testicular thermoregulation, semen quality and fertility in yearling beef bulls. It should be emphasized that infrared thermography in this study provided additional predictive fertility data among bulls that would be classified as acceptable for use as breeding sires based on the best standard breeding-soundness criteria previously available.

Acknowledgments

The assistance of Mel G. Sukup and Thomas E. Garvin in the handling of animals is acknowledged. We thank Alan J. Kruger for his technical expertise and laboratory assistance in semen evaluations.

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Table 1—Correlations between characteristics of scrotal thermograms, semen quality, testis size, and pasture-mating fertility (single-sire) among 73 yearling beef bulls[a]

	Scrotal thermogram characteristic[b]					Pregnancy
	AST	STT	STB	STG	TC	rate(%)[c]
Semen quality (n=73 bulls):						
Sperm concentration (total)	-.10	-.10	-.05	.00	.04	.16
Progressive motility (%)	.08	.03	.12	-.16	.04	-.01
Live sperm (%)	.06	.10	.04	.02	.00	.15
Normal head morphology (%)	-.07	-.13	-.10	.03	-.23**	.64***
Normal tail morphology (%)	-.12	-.20	-.07	-.07	-.18	.24
Proximal droplets (%)	.13	.17	.14	-.07	.27**	-.32*
Normal acrosomes (%)	-.06	-.03	-.09	.09	-.21*	.18
Testis size (n=73 bulls):						
Scrotal circumference (cm)	.02	.06	.04	-.01	.06	-.29
Paired testes volume (cm^3)	-.04	.00	-.02	.03	.08	-.34*
Fertility (n=30 bulls)[c]:						
No. females exposed/bull	.27	.23	.24	-.18	.03	.00
Pregnancy rate (%)	-.40**	-.33*	-.41**	.36**	-.46***	—

[a] All thermogram data was adjusted to a constant ambient temperature (50°F) before analysis. Level of significance of correlations is indicated (*P<.10, **P<.05, ***P<.01).

[b] Thermogram abbreviations: AST=average scrotal temperature, STT=temperature at top of scrotum, STB=temperature at bottom of scrotum, STG=scrotal temperature gradient, and TC=thermal class.

[c] Fertility (pregnancy) data for 30 of the 73 bulls were obtained via single-sire, natural-mating tests (18 heifers/bull, 45-day breeding period) when bulls were 15 to 17 mo of age.

Table 2—Means (± SEM) by scrotal thermogram class for semen quality, testis size, scrotal surface thermogram temperatures, and pasture-mating fertility (single-sire) among 30 yearling beef bulls[a]

	Scrotal thermogram class		
	Normal pattern	Questionable pattern	Abnormal pattern
Number of bulls	13	9	8
Breeding soundness examination (n=30 bulls):			
Total BSE score	85 ± 4	92 ± 4	92 ± 4
Semen quality (n=30 bulls):			
Sperm concentration (x 106)	981 ± 246	1205 ± 297	755 ± 302
Progressive motility (%)	64 ± 4	64 ± 5	73 ± 5
Live sperm (%)	66 ± 4	66 ± 5	70 ± 5
Normal head morphology (%)	92 ± 1	94 ± 2	91 ± 2
Normal tail morphology (%)	87 ± 4	94 ± 5	88 ± 5
Proximal droplets (%)	3.1 ± 1.1	1.2 ± 1.3	3.4 ± 1.3
Normal acrosomes (%)	80 ± 3	75 ± 4	79 ± 4
Testis size (n=30 bulls):			
Scrotal circumference (cm)	34.4 ± .6	34.4 ± .7	36.6 ± .7**
Paired testes volume (cm3)	567 ± 29	556 ± 35	695 ± 36**
Scrotal thermogram characteristic (n=30 bulls):			
Average temperature (AST,°F)	79.9 ± .6	80.6 ± .7	82.1 ± .7**
Temperature at top (STT,°F)	82.3 ± .5	81.9 ± .6	82.7 ± .6
Temperature at bottom (STB,°F)	77.1 ± .7	78.7 ± .8	81.1 ± .8***
Temperature gradient (STG,°F)	5.2 ± .4	3.2 ± .5	1.6 ± .5****
Fertility (n=30 bulls)[b]:			
No. females exposed/bull	17.7 ± .6	18.3 ± .7	17.3 ± .8
Pregnancy rate (%)	83.4 ± 3.5	85.3 ± 4.2	68.3 ± 4.3***

[a] All thermogram data was adjusted to a constant ambient temperature (50°F) before analysis. Where means for bulls with abnormal thermograms differ from means of bulls with normal and questionable thermograms, significance of the difference is indicated (*P<.10, **P<.05, ***P<.01, ****P<.001).

[b] Fertility (pregnancy) data for 30 yearling beef bulls were obtained via single-sire, natural-mating tests (approximately 18 heifers/bull, 45-day breeding period) when bulls were 15 to 17 mo of age.

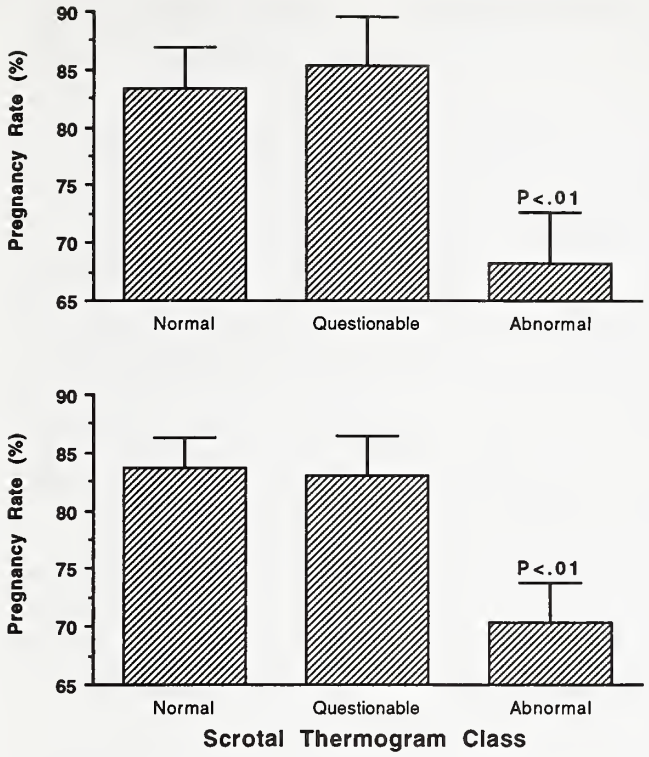

Figure 1 – Comparison of pregnancy rates achieved by yearling beef bulls (15 to 17 mo of age) classified into Normal, Questionable, and Abnormal scrotal thermograph temperature patterns. Pregnancy rates were obtained via single-sire, natural-mating exposure of each bull to approximately 18 heifers per bull during a 45-day pasture-breeding period. Thermogram data was adjusted using average ambient temperature as a covariate (upper panel) and using both ambient temperature and % normal sperm head morphology as covariates (lower panel). In both analyses, thermogram class had a significant effect on pregnancy rate and bulls with Abnormal scrotal temperature pattern exhibited reduced (P<.01) fertility, compared to the fertility of bulls with Normal and Questionable scrotal temperature patterns.

Puberty Occurs at the Same Testis Size in Both *Bos taurus* and *Bos indicus* Crossbred Beef Bulls

Donald D. Lunstra, John D. Crouse, and Larry V. Cundiff[1]

Introduction

An increasing number of *Bos indicus*-blood bulls are being used in crossbreeding programs for commercial beef production in the U.S., but there is little information available on pubertal development, sperm production and semen quality for young bulls of this type. Puberty in young *Bos taurus* bulls has been defined in a variety of ways (e.g., first ability to serve, first sperm production, first ejaculation of motile sperm, etc.), but all of these criteria are costly and labor-intensive to determine. We have shown previously (Lunstra et al., 1978) that, among young *Bos taurus* beef bulls of various breeds reared in the same environmental and management conditions, puberty occurs when a scrotal circumference of 28 cm is achieved, regardless of large differences in body wt and age at puberty among and within different breeds of bulls. In that study, puberty was defined as the age at which a bull first produced an ejaculate containing ≥ 50 million sperm with ≥ 10% progressive motility. In addition, we (Lunstra and Echternkamp, 1982) and other researchers have shown that the major characteristics of semen quality improve linearly during the first 12 to 16 wk after reaching this criterion of puberty, and that these improvements in semen quality are highly correlated (r=.44 to .75) with the steady linear increase in scrotal circumference that occurs during this post-pubertal timeframe in *Bos taurus* beef bulls. Thus, scrotal circumference appears to be an accurate and easily-obtained measurement that provides a relatively reliable predictor of age at puberty in young *Bos taurus* beef bulls. However, similar studies on *Bos indicus*-blood bulls are needed before this relationship can be assessed or confirmed in zebu-blood bulls.

It is not reasonable to attempt to use young bulls for natural mating or semen processing immediately after reaching this initial criterion of puberty (i.e., first ejaculate containing ≥ 50 million sperm with ≥ 10% progressive motility). It does not become economically feasible to use young beef bulls for collection and processing of semen for artificial insemination until ejaculates containing ≥ 500 million sperm with ≥ 50% progressive motility can be produced, and this is also a reasonable criterion for young bulls to reach before being used for natural mating. However, no studies using this more stringent semen criterion of puberty have been conducted in either *Bos taurus* or *Bos indicus* bulls. Because selection and use of superior sires at the youngest possible age is desired for natural mating and artificial insemination, further investigations of the relationships between postpubertal changes in testis size and aspects of semen quality are needed in young *Bos taurus* and *Bos indicus* beef bulls.

The following study was conducted to determine the age at which young *Bos taurus* and *Bos indicus* crossbred beef bulls reach this revised criterion of puberty (i.e., first ejaculate containing ≥ 500 million sperm with ≥ 50% progressive motility), and to evaluate interrelationships between pubertal age, testis size, and body wt in these specietypes of beef bulls.

Procedures

To evaluate age at puberty and neopubertal changes in semen characteristics, 132 spring-born bulls (80 *Bos taurus*, 52 *Bos indicus*) were evaluated monthly from approximately 8 through 20 mo of age. *Bos taurus* crossbred bulls were 3/8, 1/2 or 5/8 Hereford, Angus and Pinzgaur, and *Bos indicus* crossbred bulls were 3/8, 1/2 or 5/8 Brahman and Sahiwal (remaining fraction in each cross was Hereford or Angus for both specietypes). Bulls averaged 223 ± 1 days of age at the beginning of the study, and initial age did not differ among specietypes. All bulls had been subjected to the same managerial and environmental conditions from birth, and all had been weaned at approximately 200 days of age. Postweaning, all bulls were placed in feedlot pens (approximately 44 bulls per pen) and were fed the same growing/finishing rations throughout the study. Body wt (BWT), testis length (TL), and scrotal circumference (SCR) were measured monthly. Paired testis volume (PTV) was calculated using the formula of Lunstra et al., 1988 [PTV = 0.0396 (average TL)$(SCR)^2$]. Semen collection via electroejaculation was continued monthly until each bull first produced an ejaculate containing ≥ 500 x 10^6 sperm with ≥ 50% progressive motility (puberty). Electroejaculation was performed twice at each monthly collection date for each bull, and data for the ejaculate exhibiting the best semen quality were recorded. Semen was collected and maintained at 37°C for evaluation. Ejaculate volume was recorded, and progressive motility was determined immediately from duplicate estimates at 37°C, using a microscope (400x magnification). Sperm concentration was determined from spectrophotometer (550 nm) counts of duplicate semen aliquots diluted 1:200 with 1% formalin in 0.9% saline. Live sperm and sperm abnormalities werequantitated microscopically for each ejaculate by scoring 100 sperm in smears stained differentially with eosin-fast green.

Results & Discussion

Body wt and scrotal circumference increased linearly and continuously (P<.01) in both specietypes of crossbred bulls throughout the study (Fig. 1). Although scrotal circumference at any given age was significantly smaller in *Bos indicus* cross bulls than in *Bos taurus* cross bulls during the first 7 mo of this study, prepubertal scrotal circumference increased at the same linear rate (.040 ± .002 cm per day; r=.76, P<.01) in both *Bos indicus* and *Bos taurus* cross bulls (Fig. 1). There was no difference (P>.75; Table 1) between specietypes in scrotal circumference (32.1 ± .2 vs 32.0 ± .3 cm) at puberty (i.e., first ejaculate containing ≥ 500 million sperm with ≥ 50% progressive motility). However, *Bos taurus* cross bulls reached puberty at earlier age (P<.001; 334 ± 4 vs 404 ± 6 day) and lower body wt (P<.01; 922 ± 15 vs 1004 ± 20 lb) than did *Bos indicus* crosses. Despite these relatively large specietype differences in pubertal age and pubertal body wt, it is remarkable that bulls in both specietypes reached puberty at essentially the same testis size (Table 1).

By 20 mo of age, 100% (80/80) of *Bos taurus* cross and 83% (43/52) of *Bos indicus* cross bulls had reached puberty. A marked delay in pubertal development occurred

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only in a limited proportion of *Bos indicus* cross bulls (9/52 bulls, 17%), scrotal circumference averaged 25.5 Ò 1.3 cm at 20 mo of age in these 9 bulls, and 4 of these 9 bulls were producing spermatozoa at 20 mo of age. However, it is unknown if these bulls would have reached puberty beyond 20 mo of age. Among bulls that achieved puberty, *Bos indicus* crossbred bulls reached puberty approximately 70 days (range 56 to 95 days) later than did *Bos taurus* crossbred bulls (Table 1), but scrotal circumference did not differ (P=.75; Table 1) between these specietypes at puberty (average scrotal circumference at puberty = 32.0 ± .2 cm).

Among the breedtypes within these two major specietypes of bulls (Table 2), Pinzgaur-cross bulls reached puberty 23 to 25 days earlier than did Hereford (P<.04) and Hereford-Angus bulls (P<.06), while Angus bulls were intermediate among *Bos taurus* breedtypes. Pinzgaur-, Angus-, Hereford-, and Hereford-Angus-cross bulls reached puberty 56 to 81 days earlier (P<.001) than did Brahman and 70 to 95 days earlier (P<.001) than did Sahiwal-cross bulls (Table 2). Again, regardless of the relatively large differences in pubertal age and pubertal body wt among these breedtypes, bulls of these breedtypes did not differ (P=.22; Table 2) in scrotal circumference at puberty (average = 32.0 ± .2 cm); in other words, regardless of breedtype, all bulls reached puberty (i.e., first ejaculate containing ≥ 500 million sperm with ≥ 50% progressive motility) at essentially the same testis size.

The coefficient of variation (CV) at puberty within specietypes (Table 1) was lowest for scrotal circumference at puberty (CV = 6.6%), and the CV was much larger for age at puberty and for body wt at puberty (CV = > 14%). The dramatic variations in age and body wt at puberty are depicted graphically in Figure 2. In addition, the low variation in scrotal circumference at puberty is also shown more clearly in Figure 2. These data indicate that both *Bos taurus* and *Bos indicus* crossbred bulls reached puberty (i.e., first ejaculate containing ≥ 500 million sperm with ≥ 50% progressive motility) when testis size reached a scrotal circumference of 32 cm, regardless of relatively large specietype and breedtype differences in pubertal age and pubertal body wt. The linear correlation between a single measurement of scrotal circumference obtained in all bulls between 10 to 12 mo of age and actual age at which those bulls reached puberty ranged from r=-.65 to r=-.69 (P<.001). Given the linearity of testis development in both specietypes of bulls (Fig. 1; .040 ± .002 cm per day; r=.76, P<.01), measurement of yearling scrotal circumference appears to be an easily obtained and relatively accurate predictor of the age at which a bull will reach puberty, regardless of large specietype and breedtype differences in pubertal age and pubertal body wt.

Acknowledgments

The assistance of Mel G. Sukup, Thomas E. Garvin, James D. Ochsner, Diane R. Walters, and David J. Powell in the handling of animals is acknowledged. We thank Alan J. Kruger for his technical expertise and laboratory assistance in semen evaluations.

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Table 1—Comparison of age, body wt (BWT) and scrotal circumference (SCR) at puberty (≥500 x 106 sperm with ≥50% motility) among specietypes

		Means (at puberty)		
	n	Age (day)	BWT (lb)	SCR (cm)
All bulls	123	358	951	32.0
± SEM[a]		± 5	± 12	± .2
Specietype:				
Bos taurus	80	334	922	32.1
Bos indicus	43	404	1004	32.0
Difference[b]		P<.001	P<.001	P=.75
Coefficient of variation (%)	14.5	14.3	6.6	

[a] SEM = Standard error of the mean.

[b] Probability of a significant difference between specietypes.

Table 2—Comparison of age, body wt (BWT) and scrotal circumference (SCR) at puberty (≥500 x 106 sperm with ≥50% motility) among breedtypes

	n	Means (at puberty)		
		Age (day)	BWT (lb)	SCR (cm)
Breedtype:				
Pinzgaur (1/2,5/8)	18	318	919	31.8
Angus (5/8)	24	334	925	32.0
Angus x Hereford (1/2 x 1/2)	16	341	888	32.7
Hereford (5/8)	22	343	946	31.9
Brahman (3/8,1/2,5/8)	18	399	1018	31.9
Brahman x Sahiwal (1/4 x 1/4)	10	398	1008	33.3
Sahiwal (3/8,1/2,5/8)	15	413	985	31.2
± SEM[a]		± 9	± 30	± .5
Difference[b]		P<.001	P<.05	P=.22

[a] SEM = Standard error of the mean, averaged per breedtype.

[b] Probability of a significant difference between specietypes.

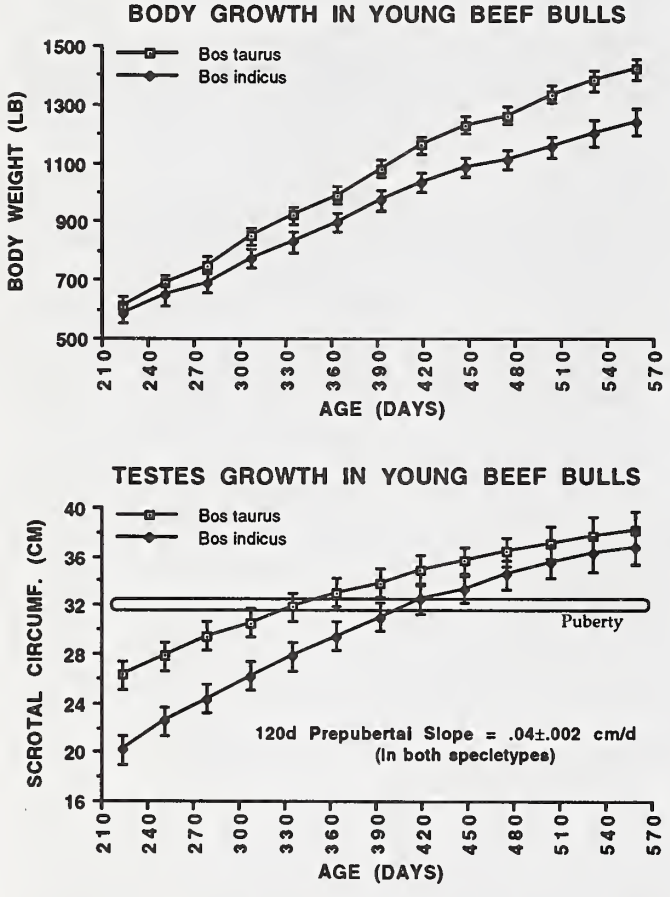

Figure 1 – Comparison of changes in body wt (upper graph) and testis size (scrotal circumference; lower graph) in *Bos taurus* and *Bos indicus* crossbred bulls during pubertal development (8 to 20 mo of age). Puberty (i.e., first ejaculate containing ≥ 500 million sperm with ≥ 50% progressive motility) occurred at a relatively constant scrotal circumference (32.0 ± .2 cm) in both specietypes of bulls. There was no difference (P>.75) between specietypes in scrotal circumference at puberty. Puberty was not related (P>.20) to bull body wt. Prepubertal scrotal circumference increased at the same linear rate (.040 ± .002 cm per day; r=.76, P<.01) in both *Bos indicus* and *Bos taurus* cross bulls.

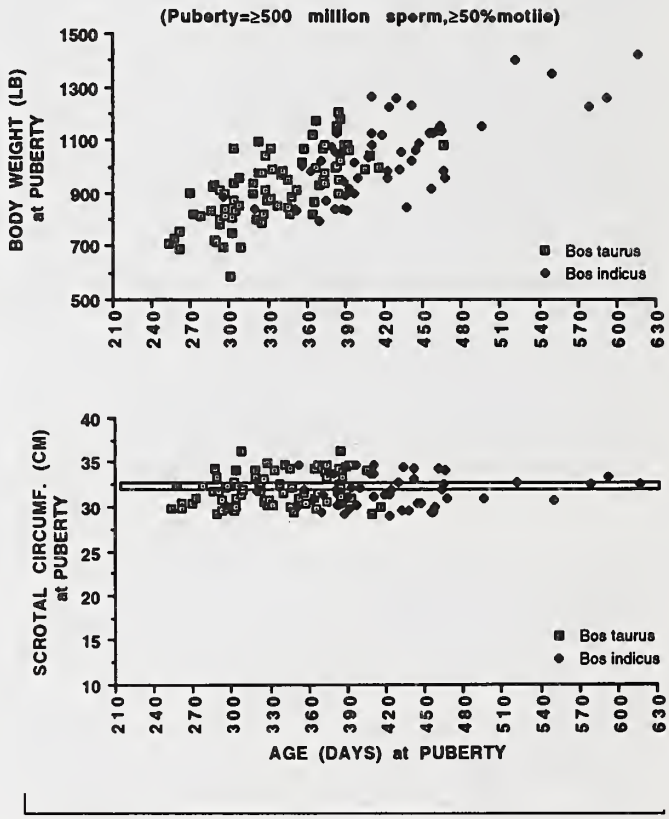

Figure 2 – Scatter graphs depicting the variation observed for body wt at puberty (upper graph) and scrotal circumference at puberty (lower graph) in *Bos taurus* and *Bos indicus* crossbred bulls that reached puberty (i.e., first ejaculate containing ≥ 500 million sperm with ≥ 50% progressive motility) between 8 and 20 mo of age. As shown in the lower graph, scrotal circumference at puberty (32.0 ± .2 cm) was relatively constant in both specietypes of bulls. There was no difference (P=.75) between specietypes in scrotal circumference at puberty. Bull body wt at puberty exhibited considerable variation and was not related significantly to age at puberty or scrotal circumference at puberty in *Bos indicus* and *Bos taurus* crossbred bulls.

Immunization Against Inhibin Increases Sperm Production in Young Beef Bulls

Donald D. Lunstra, Terry L. Martin, Gary L. Williams, and James J. Ireland[1]

Introduction

The specific biological mechanisms that control pubertal development, testicular growth and onset of sperm production in the beef bull have not been well defined. It is known that two protein hormones (the gonadotropins, LH and FSH) produced by the pituitary stimulate testicular growth, cause an increase in numbers of testicular receptors for LH and FSH, and stimulate production of steroid hormones by the testes. Researchers have shown that the patterns of LH and FSH secretion diverge during the peripubertal period in several species, including the bull. While LH increases during the peripubertal period of development in the bull, FSH exhibits little change during this period. Inhibin, a protein hormone produced by the testes, has been shown to selectively inhibit FSH secretion in other species. Thus, inhibin may explain the divergence in peripubertal patterns of LH and FSH secretion in the bull and may be an important regulatory factor in bovine testicular development.

In other species, immunization of males against endogenous inhibin has been shown to influence secretion of FSH and testicular function. Structurally, the inhibin molecule in various species is composed of two protein chains termed the alpha and the beta chains. Each protein chain is composed of numerous amino acids, but the sequence of amino acids in the alpha chain contributes to inhibin's unique hormonal properties and species specificity. Thus, immunization against inhibin is usually performed by utilizing the alpha chain to induce immunological specificity against inhibin. Immunoneutralization of endogenous inhibin has been shown to cause increased secretion of FSH in rats, and similar immunization in rams has resulted in increased FSH, LH, testis size, daily sperm output and epididymal sperm reserves. Although inhibin is present in peripheral blood of bulls, the role of inhibin in regulation of gonadal growth and function in bulls is unknown. Therefore, the objective of the present experiment was to determine the importance of inhibin in regulation of secretion of FSH, LH and testosterone, testicular growth, and/or sperm production in young beef bulls.

Procedures

Animals, Treatments and Samples– Beginning at 14 wk of age (3.5 ± 0.1 mo of age and 215 ± 9 lb body weight; mean ± SE), 20 Angus x Hereford-Brahman bulls (n = 10 per treatment group) were actively immunized against the first 26 amino acids of bovine inhibin alpha (bINH-Immun bulls) conjugated to a carrier protein, human alpha globulin (HAG), or were immunized against HAG alone (control bulls). The primary immunization was followed by booster immunizations given at 28, 30, and 34 wk of age. Body weight and scrotal circumference (an excellent indicator of paired testicular mass) were measured at the beginning of the experiment and 10 days after each immunization. A single jugular blood sample (10 ml) was collected from each bull 10 days after each booster for determination of bINH antibody titer and for hormone assays. Ten days after the last booster (at ~36 wk of age), blood was sampled at 1-hr intervals for 8 hr to quantify serum concentrations of FSH, LH, and testosterone. Bulls were castrated at 9 mo (36 wk) of age, and testicular daily sperm production was determined via homogenization.

Testicular Sperm Production– Three subsamples of testicular tissue per bull (1-2 gram subsample from the proximal, middle, and distal portions of the left testis from each bull) were obtained, and subsamples were thoroughly homogenized. The average number of homogenization-resistant spermatids per gram of testicular tissue was determined for each bull from hemacytometer counts using a microscope. To determine testicular daily sperm production (DSP), the number of homogenization-resistant spermatids per gram of testicular tissue was divided by 5.32 day (species-specific constant for calculation of daily sperm production in bulls).

Inhibin Antibody Titers– To determine the amount of anti-inhibin antibodies (i.e., bINH-antibody titer) that had been induced in each bull, blood serum was diluted 1:4000 and bINH-antibody titers were determined in all serum samples collected 10 days after each immunization. All titers were determined in one assay and the intraassay coefficient of variation (CV) was 3.7%.

Gonadotropin and Steroid Hormone Assays– Serum samples were assayed for testosterone, LH and FSH via validated laboratory radioimmunoassays. The intraassay CV was 4.4% for FSH and 4.7% for LH. Extraction efficiency for testosterone was 94%; intraassay and interassay CVs for testosterone were 3.8% and 7.6%, respectively.

Results & Discussion

Both body weight and scrotal circumference increased continuously ($P<.01$) between 14 and 34 wk of age in both treatment groups of bulls. However, body weight and scrotal circumference of treated bulls did not differ ($P>0.20$) from control bulls throughout the experiment (Martin et al., 1991). Substantial anti-inhibin titers were established in bINH-Immun bulls, and serum diluted 1:4000 from bINH-Immun bulls bound 36 ± 4%, 52 ± 5%, and 53 ± 4% radioiodinated bINH (mean ± SE of 10 bulls per group) 10 days after boosters given at 28, 30, and 34 wk of age, respectively, while binding was less than 2% in control bulls. For the single blood samples taken 10 days after the first and second boosters, serum concentrations of FSH and testosterone were similar ($P>0.20$), but concentrations of LH were decreased ($P<0.05$) in bINH-Immun compared with control bulls. However, for the blood samples obtained at hourly intervals for 8 hr (i.e., at 9 mo of age), serum concentrations of FSH were increased ($P<0.05$) substantially and serum concentrations of LH were decreased ($P<0.001$) markedly in bINH-Immun bulls compared with control bulls (Figure 1). Despite this reduction in serum LH, concentrations of serum testosterone also were increased ($P<0.05$) in bINH-Immun bulls at 9 mo of age (Figure 2). While testis size (scrotal circumference) did not differ between bINH-Immun bulls and control bulls at 9 mo of age (Martin et al., 1991), daily sperm production per gram testicular tissue was dramatically increased ($P<0.05$; Figure 2) in bINH-Immun bulls compared with control bulls.

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The threefold increase in serum FSH present at 9 mo of age in bulls immunized against inhibin, compared to control bulls, supports the concept that inhibin functions to suppress FSH in the bovine. Other research has indicated that castration of bulls causes a two- to four-fold increase in serum FSH (MacDonald et al., 1991). Although castration removes other potential FSH-regulatory factors that originate from the testis, such as steroids, our data indicate that inhibin may have a potent negative feedback effect of secretion of FSH in bulls.

Other researchers have reported that both intratesticular and blood concentrations of testosterone increase around 4-5 mo of age in bulls. High intratesticular concentrations of testosterone promote Sertoli cell differentiation, leading to differentiation of germ cells into spermatogonia and establishment and maintenance of spermatogenesis. In our study, the increased secretion of FSH could have increased testicular sensitivity to LH, since FSH increases numbers of FSH and LH receptors in testes. Despite the lower concentration of LH, testosterone concentration was greater in bINH-Immun bulls than in controls. We speculate that exposure of the testes to high concentrations of FSH may have markedly increased the sensitivity of the testes in bINH-Immun bulls to circulating gonadotropins. Therefore, increased sensitivity of the testes to gonadotropins may explain the increased secretion of testosterone, despite decreased secretion of LH, in bINH-Immun bulls.

Our most significant finding was that total daily sperm production and sperm production per gram of testicular tissue increased approximately two-fold following immunization against inhibin. This increase in sperm production coincided with increased FSH and testosterone concentrations in bINH-Immun bulls, suggesting that both hormones may enhance testicular sperm production in bulls. In bulls, testosterone is an important regulator of testicular maturation. Alternatively, inhibin has been reported to have local inhibitory effects on testicular function, and removal of inhibin via immunoneutralization may have released testicular sperm production from the local inhibitory actions of inhibin. Others have reported that inhibin decreases numbers of spermatogonia when injected into the testis of mice or hamsters. In support of this hypothesis for a localized effect of inhibin, unilateral intratesticular injections of inhibin have been shown to decrease numbers of spermatogonia in the inhibin-injected, but not in the contralateral testis, of mice and hamsters. Therefore, increased testosterone secretion and enhanced sperm production in bINH-Immun bulls may be due, in part, to removal of local inhibitory actions of inhibin on the testes.

The mechanism(s) by which inhibin immunoneutralization selectively increased spermatid density in the testis of neopubertal beef bulls is unknown. It was surprising that total daily sperm production and sperm production per gram testicular tissue was increased without significant changes in testis size (scrotal circumference). The lack of change in scrotal circumference, despite a twofold increase in spermatids in bINH-Immun bulls, may indicate that Sertoli cell function was altered and resulted in increased production of spermatids within the seminiferous tubules. In support of our findings, Schanbacher (1991) recently reported that sperm density per gram of testis, but not testicular size, tended to be increased at 13 mo of age in beef bulls that had been immunized against porcine inhibin alpha. It is possible that immunoneutralization of inhibin in young beef bulls may result in an acceleration of pubertal development and earlier onset of sperm production. Further studies are needed to determine if the increased sperm production induced by inhibin immunoneutralization continues to be maintained postpubertally in more mature bulls.

In summary, immunoneutralization of inhibin in young beef bulls increased serum concentrations of FSH and testosterone, decreased serum concentrations of LH, dramatically enhanced sperm production per gram of testis and total daily sperm production, and did not alter testis size or body weight. We conclude that inhibin plays an important role in the regulation of secretion of gonadotropins and in regulating testicular development and sperm production in young beef bulls.

Acknowledgments

We thank Alan J. Kruger for his laboratory assistance and technical expertise.

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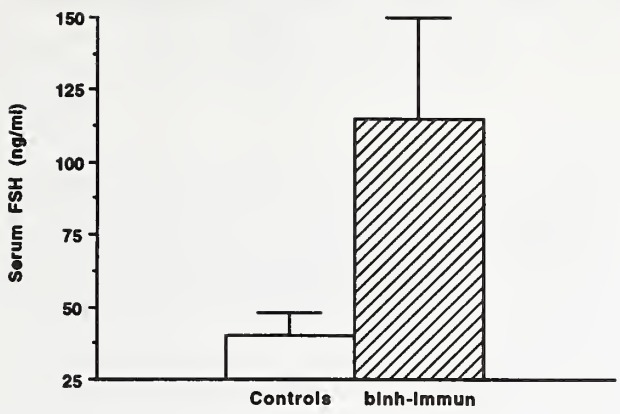

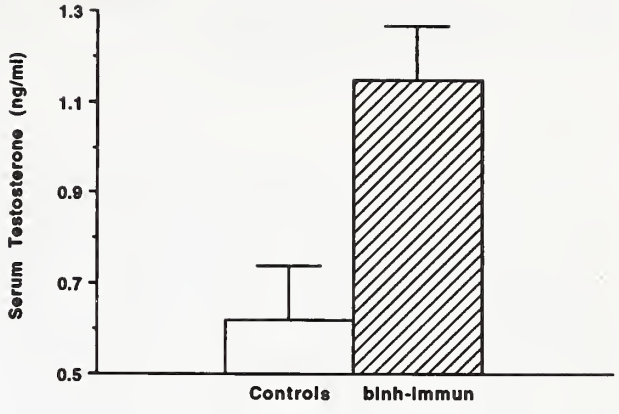

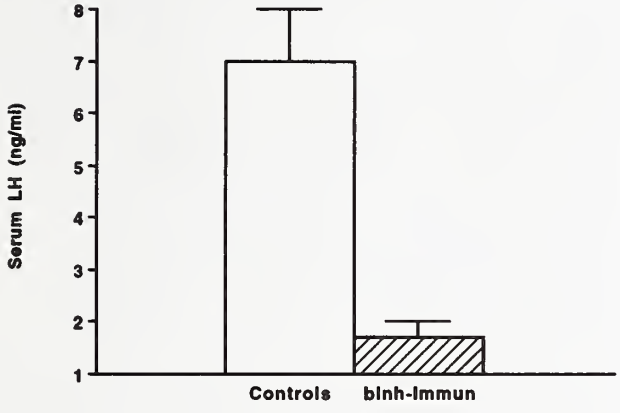

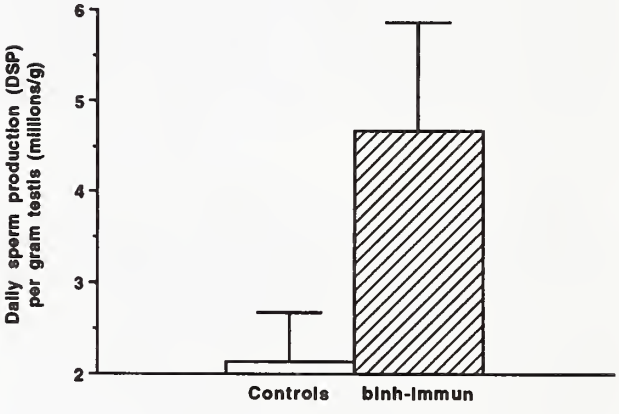

Figure 1 – Effect of active immunization against inhibin during development on serum concentrations of FSH and LH in control and bINH-Immun bulls at 9 mo (36 wk) of age. Blood was sampled at 1-hr intervals for 8 hr at 9 mo of age. Values shown are means over the 8-hr sampling period ± SE for 10 bulls per treatment group.

Figure 2 – Effect of active immunization against inhibin during development on serum concentrations of testosterone and on daily sperm production per gram of testis for control and bINH-Immun bulls at 9 mo (36 wk) of age. Daily sperm production per gram testicular tissue was calculated by dividing the number of homogenization-resistant spermatids per gram by a constant of 5.32 days. Values shown are means ± SE for 10 bulls/treatment group.

Effect of Method of Estrous Synchronization on Oocyte Quality and Follicular Insulin-Like Growth Factor (IGF-I)

Thomas H. Wise and Ralph R. Maurer[1]

Introduction

Of the two methodologies utilized in the beef industry to synchronize animals to estrus (prostaglandin regression of the corpus luteum or implanting/injecting progestin which results in estrus 48-60 hr after implant removal), conception and fertility are generally lower in progestin synchronization to estrus. Both technologies produce comparable results in relation to estrus and ovulation. Alterations in steroidal hormones of the follicle (progesterone/estradiol) are important in the maturation and quality of oocytes. Insulin-like growth factor (IGF-I) which can regulate follicular progesterone concentrations, may have a role in oocyte maturation and viability. Circulating progesterone concentrations alter luteinizing hormone (LH) pulse frequency and amplitude and possible oocyte maturation as LH is the primary hormonal initiation of the ovulatory process. The objectives of this study were to monitor the difference in oocyte quality and follicular steroids in relation to the two methods of estrous synchronization.

Procedure

Crossbred heifers were synchronized to estrus with three methods consisting of 1) prostaglandin-induced corpora lutea regression (Lutalyase, 25 mg and 10 mg, 6 hr apart, n = 30); 2) silastic progestin implants for 8 days (Norgestomet, days 7-9 of estrous cycle), which upon removal results in estrus (n = 30), and 3) prostaglandin-synchronized animals that were administered a silastic progestin implant 12 hr prior to prostaglandin injection (n = 25). All animals were superovulated with follicle stimulating hormone (FSH) administered 4, 2, 2, and 2 mg twice daily starting on day 10 of the estrous cycle. Prostaglandins were administered 60 hr after the initial FSH injection. Animals were ovariectomized (n = 5-8/time) at 12, 36, 48, 60, and 72 hr after prostaglandin injection or implant removal. Follicles were measured for size and number on the ovary, follicular fluid collected, and oocytes removed from follicular fluid. Follicular fluid was analyzed for IGF-I, estradiol, and progesterone, and oocytes were evaluated for developmental stage and quality.

Results

Analysis of follicular fluid hormones and oocyte quality (n = 1604) showed differences due to method of estrous synchronization. In Figure 1, the progesterone concentrations in small- ($\leq$ 4 mm diameter), medium- (> 4 and < 8 mm diameter), and large-size follicles ($\geq$ 8 mm diameter) of the three treatments over the estrual period are shown. There are significant differences between the prostaglandin-synchronized animals (Fig. 1a) and progestin-synchronized animals (Fig. 1b) not only in overall concentrations but also in medium- and large-size follicles in which the prostaglandin synchronized heifers have considerably more follicular progestins ($p<.01$). In the third treatment in which the LH surge was suppressed with a progestin implant administered 12 hr prior to the prostaglandin, follicular progesterone concentration remained low until 60-70 hr, indicating luteinization of unovulated follicles. Follicular estradiol changes are depicted in Figure 2. Animals receiving prostaglandin for estrous synchronization had increased estradiol concentrations, particularly at the time of the LH surge (40-60 hr), which initiates the ovulatory process and oocyte maturation (Fig. 2a). Animals that were progestin synchronized (Fig. 2b) or received a progestin implant in conjunction with prostaglandin injections (Fig. 2c) had low and comparable follicular estradiol levels. Low follicular concentrations of follicular estradiol at the time of the LH surge (40-60 hr) indicate some interference with the normal LH stimulation that accompanies follicular development/ ovulation in these two treatment groups. Follicular fluid concentrations of IGF-I decreased with an increase in follicular size and time of the estrual period ($p<.05$). IGF-I concentrations are also increased in prostaglandin-synchronized animals at 12 hr post prostaglandin as compared to progestin-synchronized animals (Fig. 3a, 3b). Differences in oocyte quality as percent degenerate are depicted in Figure 4. In the progestin-synchronized animals at 12 hr into the estrual period, 61.6 $\pm$ 4.7% of the oocytes were degenerate (Fig. 4b) as compared to prostaglandin- synchronized treatment in which only 29.9 $\pm$ 3.9% were degenerate (Fig. 4a). From 24-60 hr, all treatments were comparable in relation to oocyte viability but by 72 hr both the progestin synchronized and the prostaglandin synchronized that received a progestin implant had increased degenerate oocytes (28.0 $\pm$ 2.3 and 36.6 $\pm$ 4.2, respectively; $p<.05$).

Discussion

Differences in follicular hormonal concentrations and oocyte quality indicate that progestin-synchronized animals are responding differently at the ovarian level to the method of estrous synchronization, which may relate to differences in later fertility. Indications are that LH, a prerequisite for ovarian stimulation, steroidogenesis, and oocyte development, may be altered in progestin-synchronized animals as indicated by the lowered follicular progesterone concentrations (Fig. 1), altered estradiol concentrations (Fig. 2), and the increased numbers of degenerate oocytes detected early and late in the estrual period in progestin-synchronized animals (Fig. 4). Changes in IGF-I in progestin-synchronized animals indicate an asynchrony of the endocrine events that may also produce oocytes of poor quality and later lowered fertility.

[1]Wise is a research animal physiologist and Maurer was a research animal physiologist, Reproduction Research Unit, MARC.

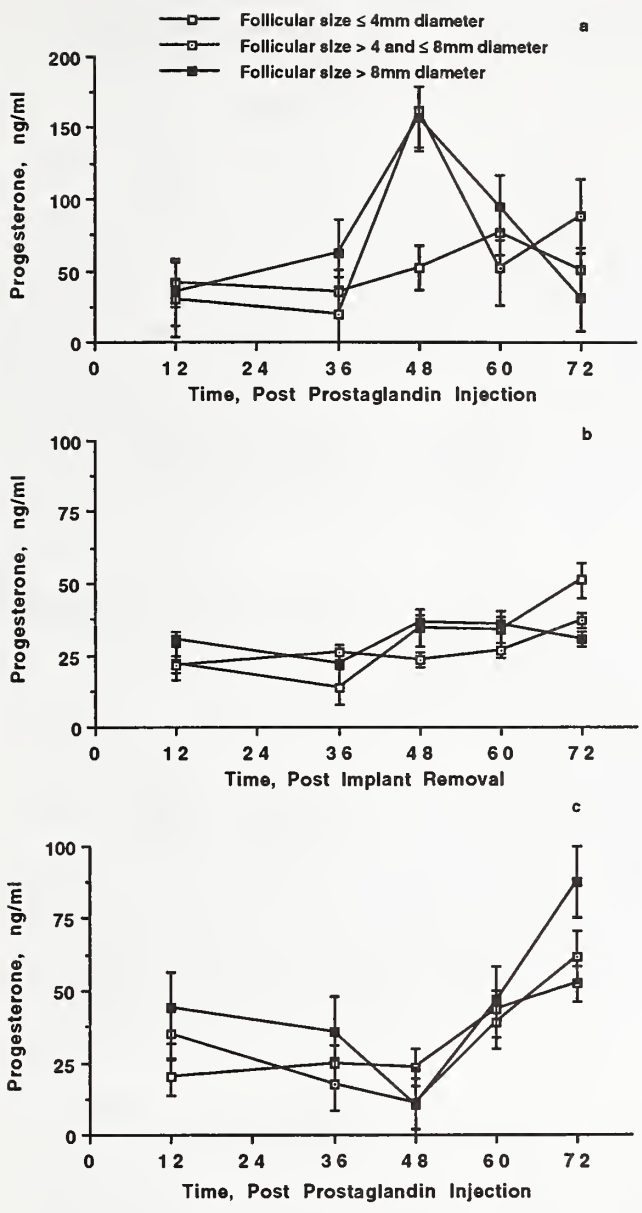

Figure 1 – Follicular fluid changes in progesterone concentration by follicle size during the estrual period in prostaglandin-synchronized animals (a), SynchroMate B progestin synchronized (b), and prostaglandin-synchronized animals that were administered a progestin implant 12 hr before prostaglandin injection (c).

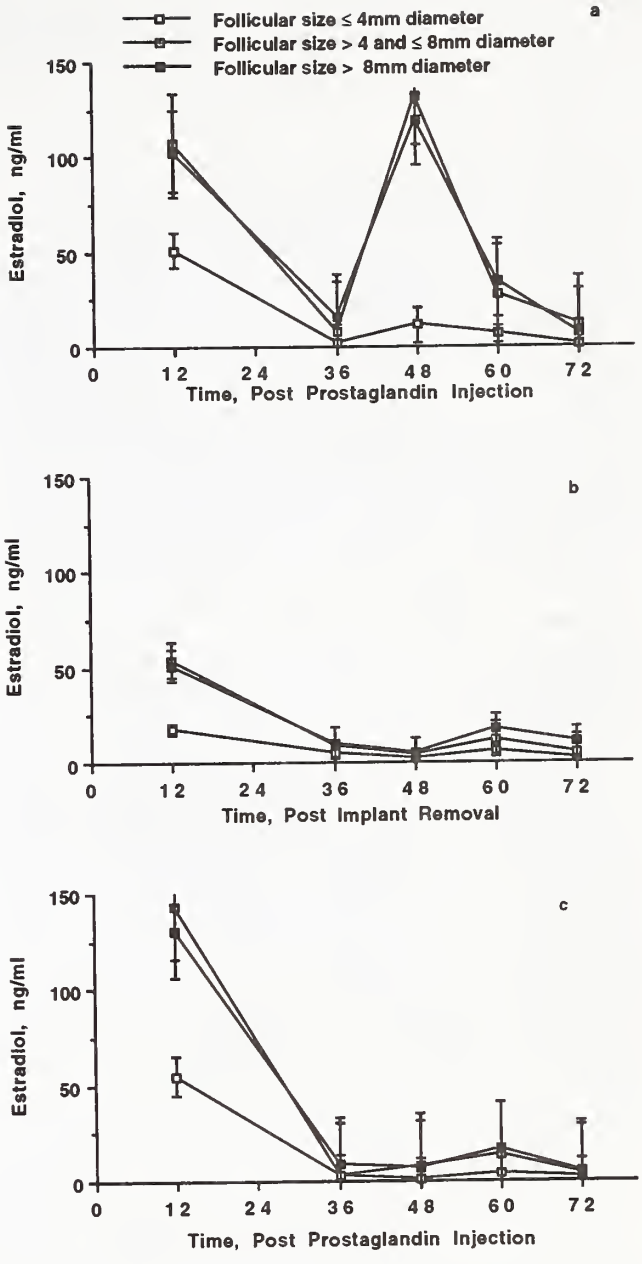

Figure 2 – Follicular fluid changes in estradiol concentration by follicle size during the estrual period in prostaglandin-synchronized animals (a), SynchroMate B progestin synchronized (b), and prostaglandin-synchronized animals that were administered a progestin implant 12 hr before prostaglandin injection (c).

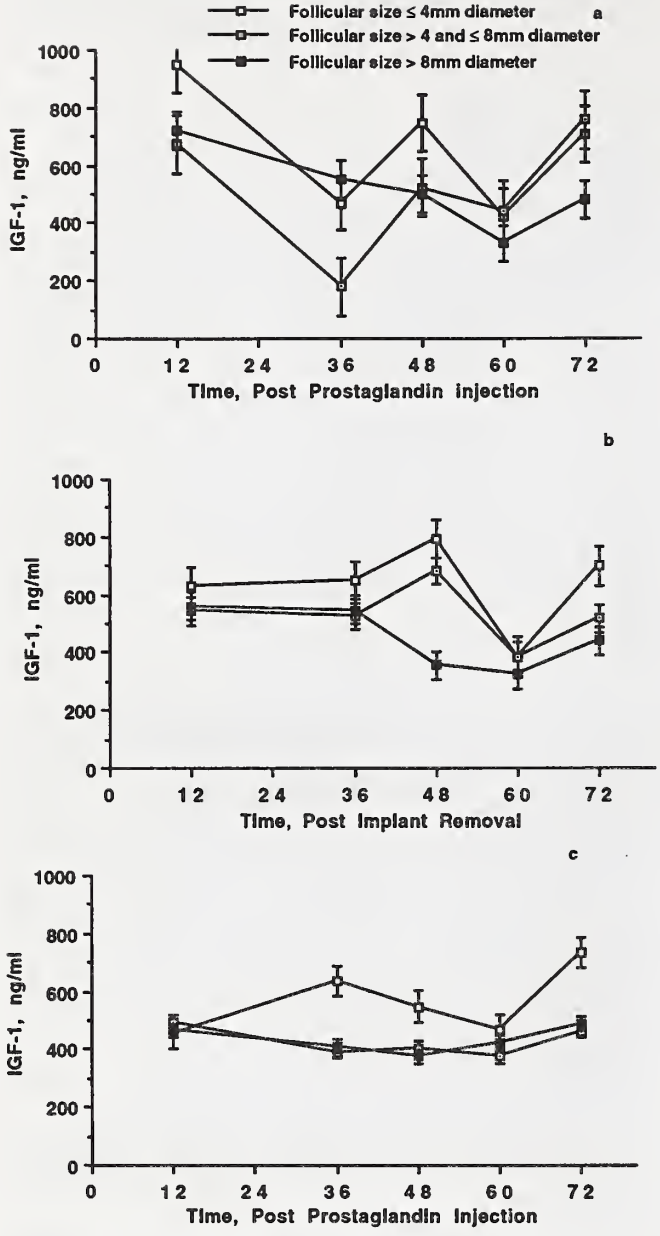

Figure 3 – Follicular fluid changes in insulin-like growth factor (IGF-I) concentration by follicle size during the estrual period in prostaglandin-synchronized animals (a), SynchroMate B progestin synchronized (b), and prostaglandin-synchronized animals that were administered a progestin implant 12 hr before prostaglandin injection (c).

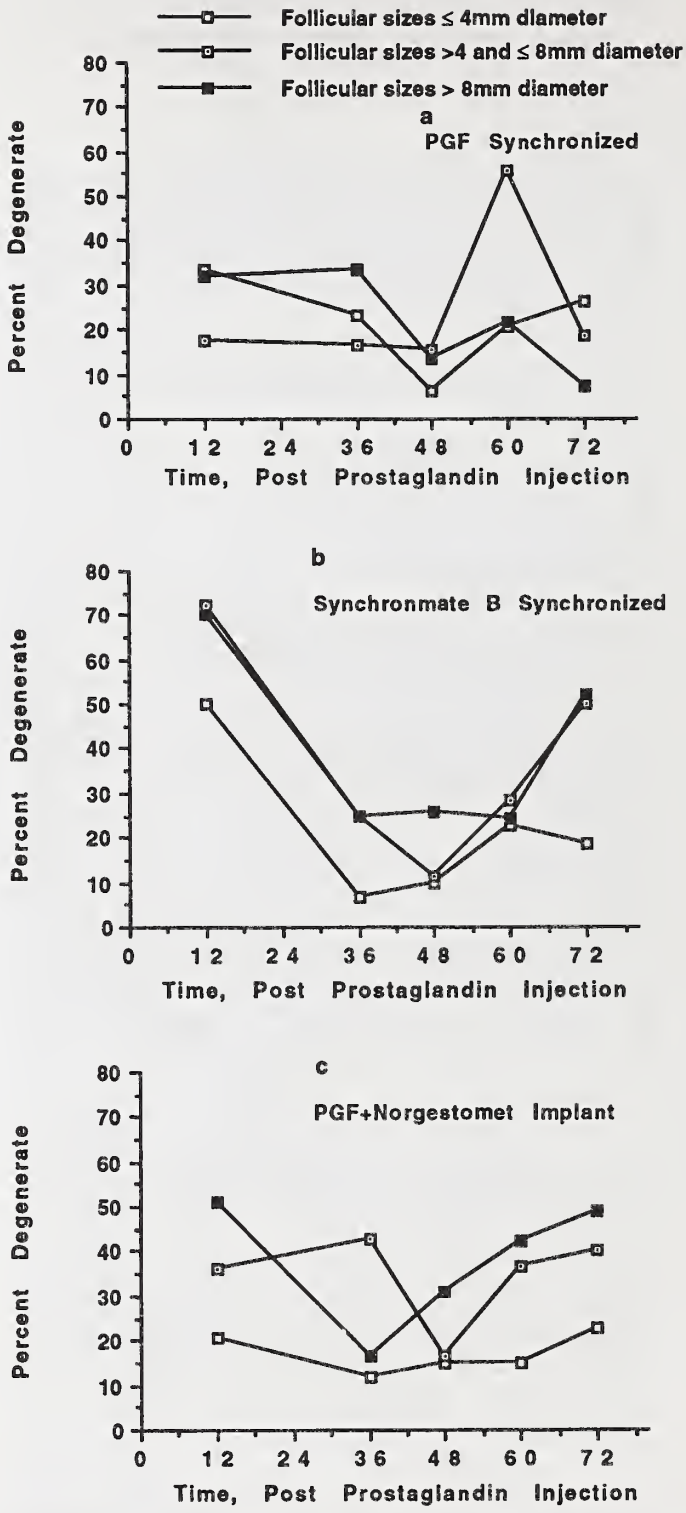

Figure 4 – Percentage of degenerate oocytes by follicular size during the estrual period in prostaglandin-synchronized animals (a), SynchroMate B progestin synchronized (b), and prostaglandin-synchronized animals that were administered a progestin implant 12 h before prostaglandin injection (c).

Follicular Hormonal Changes and Oocyte Quality in Heifers That Exhibited an LH Surge, no LH Surge, or in Which the LH Surge Was Suppressed With Progestin

Calvin L. Ferrell and Thomas G. Jenkins[1]

Introduction

The mechanisms that control follicular development, oocyte maturation and ovulation, are complex and poorly understood in farm animals. Superovulation via gonadotrophin stimulation of the ovaries provides a model to study follicular development and ovulation and the endocrine interactions at the follicle level. This study focused on the importance of luteinizing hormone (LH) in follicular development, hormonal secretion, and ovulation. The objectives of this study were to describe differences in follicular development, hormonal secretion, and oocyte quality in superovulated heifers that exhibited a normal LH surge, no LH surge, and in which the LH surge was suppressed with a progestin implant.

Materials and Methods

Crossbred heifers (n = 137) were synchronized to estrus with prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) and superovulated with follicle stimulating hormone (FSH-P). Animals were divided into three treatment groups to consist of 1) animals that exhibited an LH surge (n = 86), 2) animals that had no LH surge (n = 23), and 3) animals in which the LH surge was suppressed with a progestin implant (Norgestomet, n = 28) inserted in the ear 12 hr prior to the initial prostaglandin injection. Animals were ovariectomized every 12 hr after the prostaglandin injection (n = 7-9/time, 12-108 hr post $PGF_{2\alpha}$). Animals implanted with progestin were ovariectomized at 72, 84, 96, and 108 hr post $PGF_{2\alpha}$. Post ovulatory follicular changes involving atresia were monitored by ovariectomizing animals at 192 and 240 hr post $PGF_{2\alpha}$ (n = 34). Follicular fluid was collected after follicles were measured for size. Oocytes were centrifuged from the fluid and evaluated for viability.

Results

In the heifers that exhibited an LH surge, follicular progesterone and estradiol were increased (Figs. 1 and 2; $p<.05$), particularly at the time of the LH surge (x = 45 hr). Follicular fluid glycosaminoglycans (GAG) were increased in animals not exhibiting an LH surge, primarily in the small- and medium-size follicles (Fig. 3). Follicular progesterone and estradiol concentrations increased with follicular size whereas glycosaminoglycans decreased in concentration as follicles increased in size (Figs. 1-3). Follicular progesterone concentrations were increased in animals that did not show an LH surge as compared to the treatment group that had the LH surge inhibited with progestin implants. Follicular estradiol and glycosaminoglycan concentrations were similar in the no LH surge group and the progestin-implanted group. Oocyte recovery was 77%. Oocyte quality was poorest in small-size follicles and best in the large-size follicles (Table 1). The LH surge treatment group had the highest quality of oocytes whereas the progestin-implanted animals had the poorest quality oocytes (22% viable). Oocyte quality from follicles into the next cycle (day 4, 6) was very low (16-30% viable) and presumably indicative of aspects of follicular atresia. Estrogen and progesterone concentrations remained low in these follicles but glycosaminoglycan concentrations increased, also indicative of atresia.

Discussion

Increases in progesterone alter the release of pituitary LH and subsequently inhibit both the steroidogenic function and ovulation as indicated in the progestin-implanted animals. Also, animals in which no LH surge was detected due to handling and blood sampling during the experiment (21%) had altered steroidogenesis (Figs. 1-2), no ovulation, and decreased oocyte viability (Table 1), but peripheral circulating concentrations ot progesterone were not different from those in animals that exhibited an LH surge and later ovulation. The LH stimulation during the estrual period is also important for oocyte maturation. In animals in which the LH surge was suppressed (progestin implanted), oocyte quality was low. Animals in which no LH surge was detected had intermediate viability of oocytes, and thus probably received some LH stimulation but insufficient for complete development and ovulation. Glycosaminoglycans can be used as a biochemical marker for atresia of follicles, but also high concentrations of follicular glycosaminoglycans are related to low *in vitro* fertilization rates. High concentrations of glycosaminoglycans noted particularly in small-size follicles were related to quality (viability) of oocytes in all treatments. Further studies on the ovulatory events that related to follicular steroidogenesis, oocyte development and maturation, and ovulation will define the critical events associated with follicular development and help refine techniques that produce the maximum number of quality oocytes/embryos.

[1]Wise is a research animal physiologist and Maurer was a research animal physiologist, Reproduction Research Unit, MARC.

Table 1—Percentage of viable oocytes

Treatment	Hours post prostaglandin injection										
	12	24	36	48	60	72	84	96	108	192 (4)[a]	240 (6)
< 5 mm diameter (n = 716)											
LH surge	75.5±11.6[b]	39.9±8.6	59.2±7.8	67.9±15.8	28.5± 9.7	29.1±12.3	42.1±12.0	66.3±11.2	77.9±20.2	35.0± 8.4	1.0± 3.3
No LH surge				85.7± 4.1	52.4±18.2	90.7±16.9[c]	7.3± 9.0c	31.5±18.7[c]	37.9±12.9[c]		
Norgestomet implanted						54.4± 5.8[c,d]	37.6± 5.7d	21.4± 5.6[c]	24.2± 6.3[c]		0.0
5-8 mm diameter (n = 441)											
LH surge	54.9± 6.6	65.7±8.2	29.3±8.7	67.8±12.5	84.6± 9.8	97.2±10.2	80.9±12.1	70.9±13.8	17.4±19.6	24.0±12.6	23.3± 9.1
No LH				28.6±16.6[c]	57.6±16.2	68.0±14.5	63.5±14.4[b]	69.8±18.4	65.7±11.9		30.0±24.3
Norgestomet implanted						36.7± 6.7[c,d]	29.2± 7.0[c,d]	22.5± 7.2[c,d]	20.8± 6.0[c,d]		
> 8 mm diameter (n = 528)											
LH surge	53.6± 7.1	57.7±5.5	82.6±8.3	67.8± 9.7	66.6± 5.4	96.6±13.8	98.7±20.3	70.5±22.9	17.4±38.0	5.6±29.0	11.3± 9.4
No LH				68.5±10.8	76.6± 9.7	72.2±14.3	22.9±13.3[c]	56.4± 9.0	18.5±26.0		100.0
Norgestomet implanted						16.7± 7.5[c,d]	19.3± 5.6[c]	19.7± 7.8[c,d]	19.5± 6.4		

[a] Day of subsequent estrous cycle ( ).

[b] Time 12-36 hr same for LH and no LH surge groups.

[c] LH vs no LH or Norgestomet implanted, p<.05.

[d] No LH vs Norgestomet implanted, p<.05.

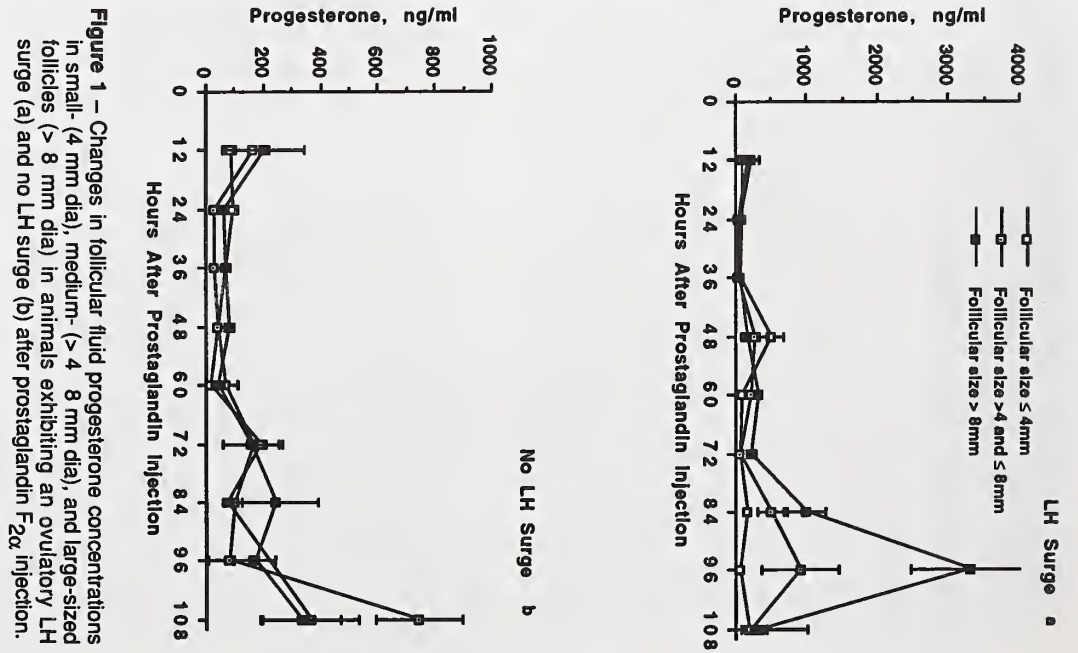

Figure 1 – Changes in follicular fluid progesterone concentrations in small- (4 mm dia), medium- (> 4 8 mm dia), and large-sized follicles (> 8 mm dia) in animals exhibiting an ovulatory LH surge (a) and no LH surge (b) after prostaglandin $F_{2\alpha}$ injection.

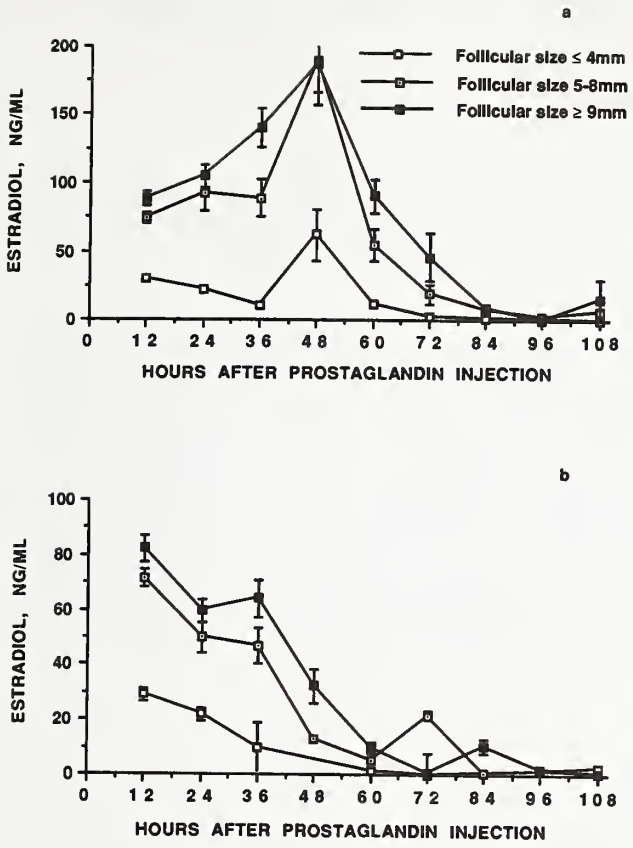

Figure 2 – Changes in follicular fluid estradiol concentrations in small- (4 mm dia), medium- (> 4 8 mm dia), and large-sized follicles (> 8 mm dia) in animals exhibiting an ovulatory LH surge (a) and no LH surge (b) after prostaglandin $F_{2\alpha}$ injection.

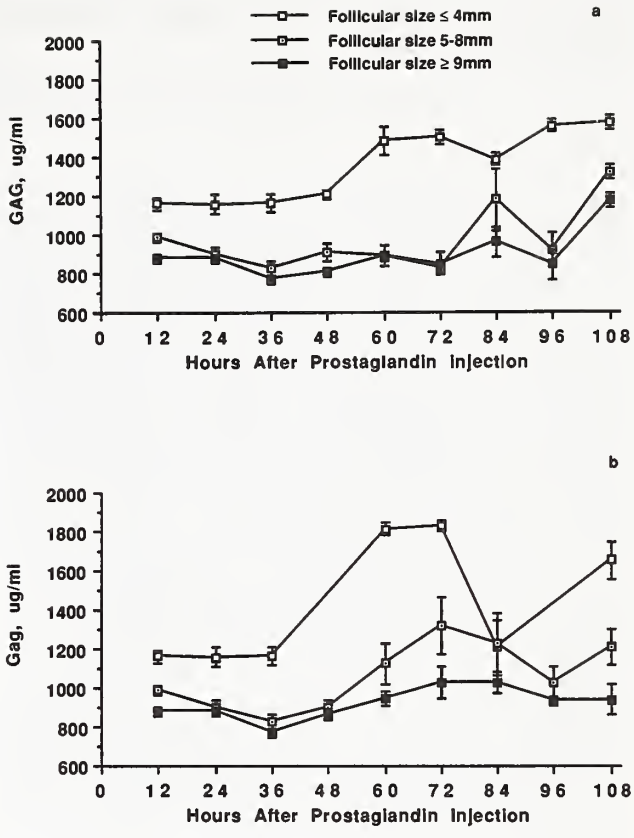

Figure 3 – Changes in follicular fluid glycosaminoglycans (GAG) concentrations in small- (4 mm dia), medium- (> 4 8 mm dia), and large-sized follicles (> 8 mm dia) in animals exhibiting an ovulatory LH surge (a) and no LH surge (b) after prostaglandin $F_{2\alpha}$ injection.

Relationships of Thymic Peptides Thymosin α_1 and β_4 With Reproductive Status: Puberty and Estrus

Thomas H. Wise, Michael L. Day, James E. Kinder, and Ralph R. Maurer[1]

Introduction

The thymus gland has been analyzed in depth in relation to its immunological function, but new evidence is accumulating that the thymus and its endocrine secretions (interferons, interleukins, thymic peptides) may be required for normal reproduction. The thymus gland regresses at puberty and pregnancy, indicative of the effect of gonadal steroids on the gland. Steroidal interactions have been hypothesized to mediate the secretion of some thymic peptides. Thymosin β_4, a thymic peptide, seems to have an integrative role in gonadal function by promoting the release of gonadotrophin releasing hormone whereas thymosin α_1 causes the release of adrenocorticotrophin releasing factor and stimulation of the adrenal gland. Evidence from rodents has established a strong role for the thymus and its secretions in reproduction, but thymic-gonadal relationships are unknown in farm species. The puberal period of development when the thymus regresses and ovarian function is initiated and the estrual period when there are maximum changes in ovarian hormones were utilized as models to monitor for relationships of thymic secretory peptides and reproductive function. The objectives of this study were to identify changes in thymic secretory peptides thymosin $\alpha_1$and β_4 during the prepuberal period of development and during the estrous period.

Materials and Methods

The first experiment analyzed changes in thymosins as related to luteinizing hormone (LH) during the prepuberal period and some of the mechanisms that may relate to LH and thymosin secretion. Sixteen heifers (Angus x Hereford; 266 ± 3 days of age) were divided into three treatments: 1) controls (n = 6), 2) ovariectomized (n = 5), and 3) ovariectomized that received an estradiol implant that would suppress increases in LH (n = 5). Sequential blood samples were acquired on days 0, 8, 36, 50, 64, 78, 92, 106, 120, and 134. Control animals were expected to attain puberty approximately 150 days after the initiation of the experiment.

In a second experiment, 103 crossbred heifers were synchronized to estrus and superovulated with follicle stimulating hormone (FSH). Twenty- eight animals were administered a progestin implant 12 hr prior to the initial prostaglandin injection. The progestin implant would effectively suppress pituitary release of LH and inhibit ovulation and alter follicular steroid secretion in this treatment group. The FSH-stimulatory regime would provide maximum stimulation to ovaries and response to estradiol secretion. Animals were divided into three treatment groups to consist of 1) animals that exhibited an LH surge (n = 56), animals in which no LH surge was detected due to stress of sampling (n = 19), and those that had the LH surge suppressed with a progestin implant (n = 28). Blood samples were drawn from the tail vein at 12-hr intervals prior to the initial prostaglandin injection and every 6 hr thereafter until 108 hours. Blood samples were analyzed for thymosin α_1, thymosin β_4, and LH by radioimmunoassay.

Results

In Experiment 1, puberty occurred in the control heifers 122 ± 10 days as determined by increased progesterone concentrations in the blood indicating a functioning corpus luteum. In control heifers, thymosin β_4 concentrations decreased in concentration until day 92, followed by a twofold increase by puberty (388 days of age) and serum LH gradually increased until puberty (Fig. 1a). In ovariectomized heifers, feedback effects of the gonads are eliminated and LH quickly increased, but thymosin β_4 changes were comparable to controls (Fig. 1c). In ovariectomized heifers receiving estradiol implants, LH was depressed and, as estradiol gradually cleared, LH increased with time (Fig. 1b). No differences were noted in the thymosin β_4 changes in estradiol-implanted animals.

In Experiment 2, thymosin β_4 and α_1 concentrations increased with time and decreased after the time of the LH surge. In both groups that had no LH surge, a spike of thymosin β_4 was noted around 100 hr (Fig. 2a). Thymosin β_4 was lower in animals with progestin implants (Fig. 2a), and thymosin α_1 was lower in animals that had no LH surge (Fig. 2b).

Discussion

Concentrations of thymosin β_4 do change during the development of the prepuberal heifer but had no relationships related to the treatments (ovariectomized or ovariectomized plus estradiol implant) in which both ovarian and pituitary hormones were altered. Changes in thymosin β_4 were similar for all treatments in Experiment 1 and suggest an alternative control mechanism outside gonadal inputs during the prepuberal period.

In Experiment 2, both thymosin hormones increased by changes that accompany the estrual period. Elevated concentrations of estradiol from the superovulatory procedure had no effect on thymosin changes. Progestin implants did depress thymosin β_4 but had no effect on thymosin α_1. Thymosins were increasing during the period of FSH injections for the superovulatory stimulation and decreasing after FSH injections ceased, and thus may be under the control of pituitary gonadotrophins. The immunosuppressive effects of progesterone and decreased levels of thymosin β_4 noted during the estrual period in implanted animals may be related to the depressed fertility noted when progestins are utilized to synchronize estrus in heifers for artificial insemination/embryo transplant studies. Changes noted in thymosin α_1 and β_4 support an endocrine interaction with the immune and reproductive system during estrus.

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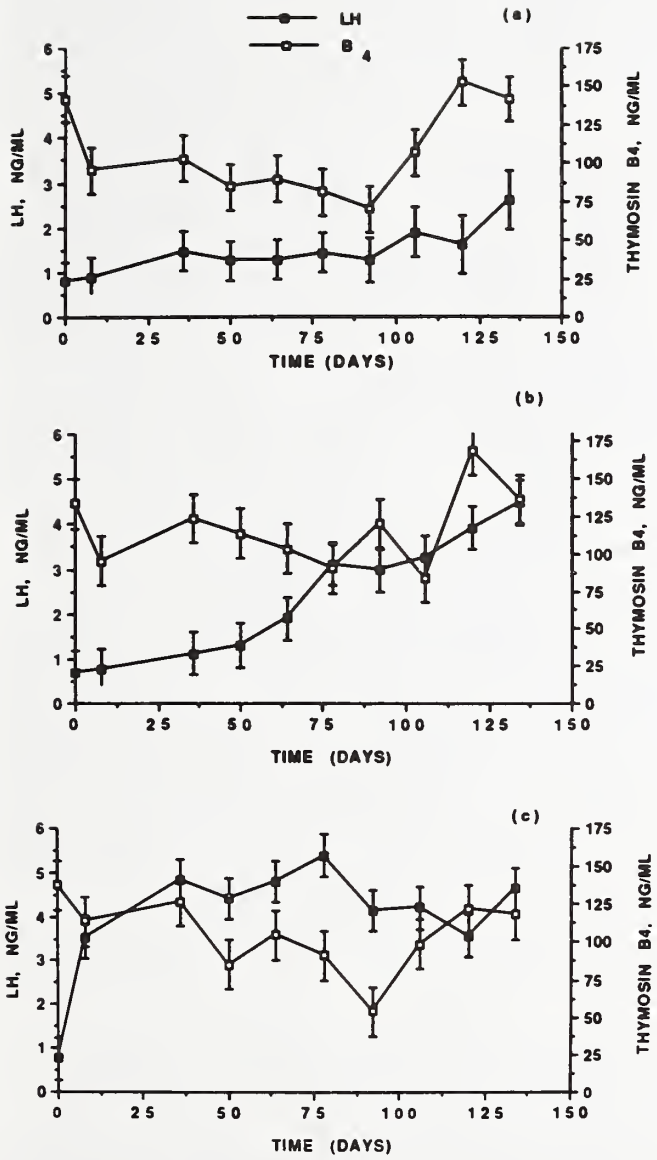

Figure 1 – Changes in luteinizing hormone (LH) and thymosin β_4 (B_4) in control heifers (n = 6, a), ovariectomized heifers administered estradiol implants (n = 5, b), and ovariectomized heifers (n = 5, c) throughout the prepubertal period. Control animals attained puberty 122 ± 10 days after the initiation of the experiment (day 0).

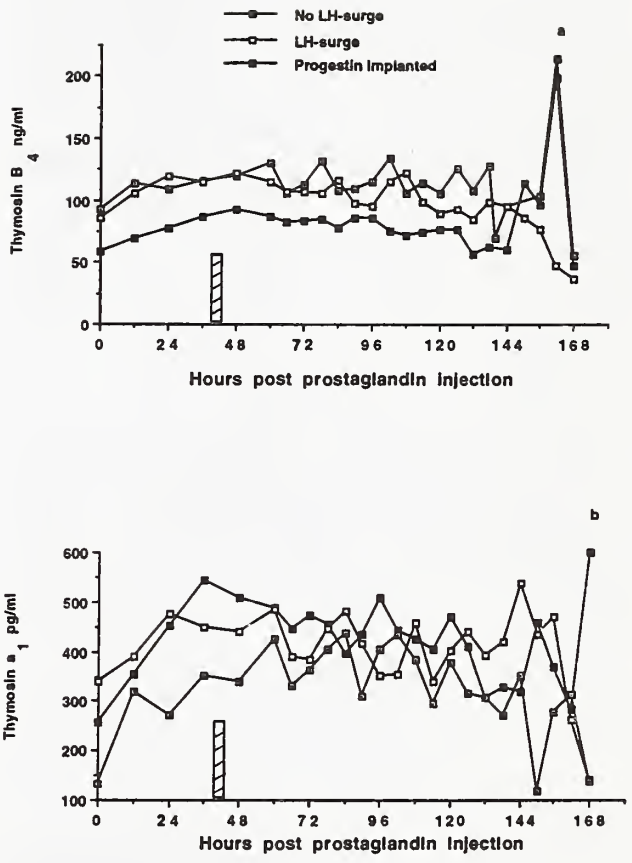

Figure 2 – Changes in thymosin β_4 (a) and thymosin α_1 (b) in superovulated heifers that exhibited an LH surge, no LH surge, and those in which the LH surge was suppressed with a progestin implant. Time 0 = initial prostaglandin injection to initiate luteal regression. Bar represents average time of LH surge in that treatment group.

Factors Influencing Fetal Growth and Birth Weight in Cattle

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Introduction

Fetal growth, as indicated by birth weight, has important influences on animal production. Birth weights lower than optimum are associated with reduced energy reserves, lowered thermoregulatory capability, and increased calf deaths at or near birth. In addition, low birth weights are related to low rates of growth after birth and decreased mature size. Conversely, birth weights greater than optimum are associated with greater calving difficulty. Primarily because of the increased calving difficulty, calf losses at birth and difficulties if rebreeding the cow are increased.

Fetal growth, hence birth weight, is influenced by numerous factors including number of fetuses, sex, parity or age of the cow, breed of sire, breed of dam, heat or cold stress, and nutrition. The importance of these and other effectors of fetal growth vary. In general, however, birth weight of each fetus decreases with increased numbers of fetuses, is greater for males than for females, and increases with age or parity of the cow. Birth weights are decreased by heat or inadequate nutrition, and increased by cold. Both the sire and dam contribute to differences in genetic potential for growth, but it is evident that the dam exerts an influence beyond her contribution to fetal genotype.

It is important to know what the factors are that affect fetal growth and their potential magnitude. In order to minimize adverse effects of factors influencing fetal growth, it is perhaps more important to understand how and why differences in fetal growth and birth weight occur. The following sections will summarize several experiments conducted to develop a better understanding of some of the major factors affecting fetal growth.

Experimental Procedures and Results

Experiment 1. The purpose of this study was, in part, to determine the influence of nutritional status of cows of different breeds on calf birth weight. Mature Angus, Braunvieh, Charolais, Gelbvieh, Hereford, Limousin, Pinzgauer, Red Poll, and Simmental cows were fed individually in dry lot for four years. Four cows of each breed were fed a ground alfalfa hay based diet at each of four feed levels (low=130, medium=170, high=210, and very high=250 kcal metabolizable energy per kilogram initial body size). Feed levels were adjusted upward during lactation to maintain body weight. Cows were bred to bulls of the same breed during a 90-day breeding season each year. Cows were calved on pasture and returned to the drylot barns within 14 days after calving. Calves were weighed within 24 hr after birth.

Calf birth weights differed substantially among the nine breeds of cattle (Table 1). Average weights were: Angus 76.2 lb, Braunvieh 111.7 lb, Charolais 104.2 lb, Gelbvieh 94.9 lb, Hereford 79.5 lb, Limousin 92.4 lb, Pinzgauer 102.8 lb, Red Poll 82.1 lb, and Simmental 106.2 lb. Observed birth weights were similar to those reported from the much larger Germplasm Utilization Project for these breeds of cows. Weights of calves from cows on the low feed level averaged 86.3 lb, whereas those from the medium, high, and very high averaged 96.9, 96.1, and 98.4 lb, respectively. Nutritional effects were much less than the breed effects and were, in general, larger in magnitude in breeds having larger calves. It is suggested that low levels of maternal nutrition may result in reduced birth weight, but nutritional levels above adequate result in no further increase.

Experiment 2. The purpose of this study was to determine rates of energy and nutrient use by the fetus and uteroplacenta (uterus + placenta) and to determine how these variables change as gestation advances. Mature Hereford cows were fed a corn silage based diet to maintain body weight and were bred to Simmental bulls. Catheters were surgically implanted in a uterine artery, a uterine vein, a fetal artery and vein, and an umbilical vein on 132, 176, 220, or 245 days after mating. Rate of maternal blood flow to the uterus (uterine blood flow) was determined four to six days after surgery. Concentrations of oxygen, glucose, lactate, and alpha-amino nitrogen (AAN, a measure of total amino acids) in samples from the uterine arterial (A) and venous (V), umbilical venous (v), and fetal arterial (a) catheters. Concentration differences (A-V and v-a) were calculated. Net uterine uptake of each metabolite was calculated as uterine blood flow X A-V and net fetal uptake was calculated as umbilical blood flow X v-a. Uteroplacental uptake was calculated as uterine-fetal uptake.

Uterine blood flow increased about 4.5-fold during the interval from 137 to 250 days, whereas umbilical blood flow increased 21-fold during this interval (Table 2). Neither metabolite concentrations nor concentration differences changed during this interval. It is important to note, however, that umbilical venous oxygen concentration was 67% of uterine arterial concentration (4.25 vs 6.36 mM), and umbilical glucose concentration was 48% of uterine arterial glucose. Conversely, umbilical AAN was 169% of uterine arterial concentrations. These results agree with data reported previously indicating oxygen and glucose are transported to the fetus by "facilitated diffusion." Amino acids are transported by active transport mechanisms. As a result, the fetus is less susceptible to changes in amino acids (protein) than to changes in oxygen or glucose.

Since neither metabolite concentrations nor concentration differences changed during gestation, changes in net uptake of those metabolites during gestation (Table 3) primarily reflect changes in uterine and umbilical blood flows. Net oxygen, glucose, and AAN uptakes by both the gravid uterus and fetus increased several fold during this interval of gestation, but fetal uptakes increased more rapidly than gravid uterine uptakes. Proportions used by the fetus generally increased over time, but the fetus used only 20 to 55% of oxygen, 3.5 to 17% of glucose, and 35 to 78% of AAN taken up by the gravid uterus. The difference was used by uteroplacental tissues. These results indicate a very high rate of nutrient and energy use by uterine and placental tissues, especially during earlier stages of gestation.

Experiment 3. This study was designed to evaluate the effects of chronic environmental heat stress on gravid uterine and fetal metabolism. Mature Hereford cows were bred to Simmental bulls, then assigned to control or heat stress treatments on day 100 of gestation. Control cows were maintained in a barn at 60 degrees and heated cows were maintained at 97 degrees, 50% relative humidity for 12 hr and 82 degrees, 50% relative humidity for 12 hours. All

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cows were fed a corn silage based diet to maintain maternal body weight. Catheters were surgically implanted (day 163) as described for Experiment 2. Uterine and umbilical blood flows and nutrient uptakes were determined as described on day 170. Fetuses were surgically removed on day 174.

Fetal weights (Table 4) were reduced 18% by the heat treatment. Uterine and umbilical blood flows were reduced 34% and 23%, respectively. Reduced uterine blood flows were likely a result of increased blood flow to the lungs and skin to facilitate heat dissipation. Neither metabolite concentrations nor concentration differences differed much between control and heated cows. Gravid uterine, fetal and uteroplacental uptakes of all metabolites (Table 5) were reduced in heated cows. The results indicated that metabolism of uteroplacental tissues was more adversely affected than was the fetus.

Experiment 4. The purpose of this study was to determine the effects of cow breed and number of fetuses on gravid uterine and fetal metabolism. Mature Charolais (bred to Charolais bulls) and Hereford cows (bred to Gelbvieh bulls) carrying either single or twin fetuses were used. Catheters were implanted (day 177) into each fetus and gravid uterine horn and measurements were made (day 183) as previously described.

Uterine blood flow was about 50% greater in Charolais (7.07 liter/min) than in Hereford (4.80 liter/min) cows (Table 6). Uterine blood flow per fetus was reduced about 20% in both Hereford cows carrying twin as compared to single fetuses. These findings indicated those breeds of cows responded similarly to twin pregnancies. They also showed that the difference due to cow breed was larger than the difference due to twins. Umbilical blood flows were similar in Charolais (1.17 liter/min) and Hereford (1.23 liter/min) cows. This result likely reflects similarity in fetal size at this stage of gestation. The ratio of umbilical to uterine blood flows for Hereford (.256) was greater than for Charolais (.166) cows. This observation indicates a greater potential for maternal constraint of fetal growth in late gestation in Hereford than in Charolais cows. Averaged across all cows, umbilical blood flow per twin fetus was reduced about 20% as compared to single fetuses, but the reduction appeared to be greater in Hereford cows. Other MARC data indicated birth weights of Charolais calves were about 24% heavier than Hereford calves. Calves born as singles were 16% heavier than calves born as twins, when weights were adjusted to equal gestation length, but the difference was about 24% when data were not adjusted. As in previous studies, net metabolite uptakes primarily reflected differences in blood flows (data not shown).

Experiment 5. The purpose of this study was to quantify maternal and paternal influences on rates of nutrient supply to uteroplacental tissues and the fetus. Mature, multiparous Gelbvieh and Pinzgauer cows mated to either Charolais or Longhorn bulls were used. Catheters were implanted (day 220) and measurements were made (days 227 and 241) as described for Experiment 2.

Both uterine (36%) and umbilical (26%) blood flows (Table 7) were greater in cows with Charolais-sired than in those with Longhorn-sired fetuses. Uterine blood flows were 18% greater in Pinzgauer than in Gelbvieh cows, but did not measurably change from 227 to 241 days. Umbilical blood flow was similar in Gelbvieh and Pinzgauer cows, but increased from 227 to 241 days. Oxygen uptakes by gravid uterine tissues were about 30% greater in Pinzgauer than in Gelbvieh, but fetal uptakes were similar. These data indicated greater rates of metabolism of uterine and placental tissues in Pinzgauer than in Gelbvieh cows. Uterine and fetal oxygen uptakes were greater in cows with Charolais-sired than in cows with Longhorn-sired fetuses. In total, these data indicated that uterine blood flow, hence nutrient to the gravid uterus and placental metabolic activity, was influenced by growth potential of the fetus as well as cow breed.

Experiment 6. Objectives of this experiment were to evaluate maternal and fetal influences on growth and metabolism of gravid uterine tissues of the cow. Brahman cows with Brahman or Charolais fetuses and Charolais cows with Brahman or Charolais fetuses (these combinations were produced by embryo transfer) were used. Catheters were surgically implanted in half of the cows at 220 days and measurements were taken at 227 days as described previously. Those cows were killed at 232 days of gestation. The other half of the cows were killed at 271 days after mating. Weights of fetuses and uteroplacental tissues were determined at slaughter.

Uterine blood flow in Brahman cows at 232 days was not affected by fetal breed (avg 4.8 liters/min, Table 8), even though Charolais fetuses weighed nearly twice as much as Brahman fetuses (Table 9). Uterine blood flow in Charolais cows was much greater than in Brahman cows. In addition, uterine blood flow in Charolais cows was greater (29%) when they were carrying Charolais fetuses than when they were carrying Brahman fetuses. These observations suggest that Brahman and Charolais cows responded differently to stimuli from the fetus or conceptus and that as a result, maternal perfusion of the uteroplacental tissues differed substantially. Umbilical blood flow was 68% greater for Charolais than for Brahman fetuses. This value was similar to the 77% difference in fetal weight. These results suggest that umbilical blood flow primarily reflected fetal weight.

Fetal oxygen uptakes paralleled fetal weight and umbilical blood flows. However, oxygen use by uteroplacental tissues did not reflect fetal, uterine or placentome weight. Further, the low oxygen use by uteroplacental tissues in Brahman cows with Charolais fetuses indicated oxidative metabolism by those tissues was substantially reduced in those groups. In contrast, glucose uptakes were greatest for Charolais fetuses in Charolais cows, intermediate for Charolais fetuses in Brahman cows, and least for Brahman fetuses. Uteroplacental glucose use was greater for Charolais than for Brahman fetuses, but not substantially altered by cow breed. These results may be interpreted to indicate that the fetus had higher priority for oxygen, but that the uteroplacenta had higher priority for glucose. The pattern of AAN use by fetuses was similar to that of glucose, but a much higher proportion was degraded for use as an energy substrate to compensate for the low glucose uptake by Charolais fetuses in Brahman cows (data not shown). Overall, the data indicated that both fetal and uteroplacental metabolism was altered a great deal, especially in the Brahman cows with Charolais fetuses, presumably to compensate for the low uterine blood flow and the resulting low rates of nutrient delivery to the gravid uterine tissues.

The influence of both cow and fetal breed on fetal growth is shown in Table 9. At 232 days, fetal weight was clearly affected by fetal breed but not by cow breed. During the ensuing 39 days, however, Charolais fetuses in Charolais cows gained 1.38 lb/day whereas fetuses of the same breed in Brahman cows gained only .58 lb/day. It is evident that in this comparison, fetal growth was constrained in the Brahman cow. Similarly, Brahman fetuses gained .99 lb/day in Charolais cows but .70 lb/day in Brahman cows. This result suggests that fetal growth may be limited by the maternal system, even in the "normal" situation.

Discussion

It is evident from the information presented, as well as from other information available, that the primary contributor to differences in fetal growth is fetal genotype, which consists of contributions from both the sire and dam. In essence, fetal genotype determines the maximum potential for fetal growth. However, it may be argued that the fetus rarely expresses its full genetic potential for growth. The cow, through her "uterine environment," may limit fetal growth to varying degrees as shown in Experiment 6. As a result, the cow's contributions to fetal growth and birth weight extend beyond her contribution to fetal genotype. Numerous other factors including maternal nutrition, number of fetuses, and environmental temperature may cause further limitation of fetal growth. These effects are most apparent during the latter stages of gestation when fetal growth rate and nutrient needs are the greatest.

The effects of many of the factors affecting fetal growth appear to be mediated, either directly or indirectly, through the nutritional status of the fetus. In this regard, nutrient availability to the fetus may be altered by nutritional status of the mother, or by other factors such as the maternal uterine environment, heat stress or increased numbers of fetuses. Uterine blood flow and function of the uterus and placental tissues appear to be major factors involved, because they are responsible for nutrient delivery to the gravid uterus and to the fetus. Uterine blood flow and function of the uterus and placenta are affected by various "external" factors such as environmental temperature; the amount of change depends on the nature, severity, duration, and timing of external stress. They are also affected by several "internal" factors such as the fetal genetic potential for growth, number of fetuses, and the cow's ability to respond to fetal needs. It is evident that the ability of the cow to respond to fetal stimuli is also under genetic control. What the primary signals from the fetus are and what mediates the cow's response to those signals are not known.

Table 1—Influence of breed and cow nutritional status on calf birth weight (lb)

	Nutritional level			
Breed	Low	Medium	High	Very high
Angus	71.4	78.3	80.7	74.3
Braunvieh	98.1	106.7	127.9	114.0
Charolais	82.0	105.6	106.3	122.8
Gelbvieh	86.6	98.8	95.7	98.5
Hereford	75.0	84.4	78.9	79.8
Limousin	86.6	93.5	94.8	94.8
Pinzgauer	100.3	113.8	93.7	103.4
Red Poll	79.8	86.4	81.4	80.7
Simmental	97.0	104.3	105.6	117.7

Table 2—Uterine and umbilical blood flows at different stages of gestation

	Day of gestation			
	137	180	226	250
Uterine blood flow, liter/min	2.92	4.78	8.75	13.18
Umbilical blood flow, liter/min	0.28	1.07	2.79	5.86

Table 3—Net uptakes of metabolites by the gravid uterus and fetus at different stages of gestation[a]

		Day of gestation			
		137	180	226	250
Oxygen	Gravid uterus	2.02	3.13	7.15	12.53
	Fetus0.40	1.50	4.18	6.92	
Glucose	Gravid uterus	0.57	0.84	1.38	2.60
	Fetus	0.02	0.10	0.24	0.42
AAN[b]	Gravid uterus	0.66	1.35	3.22	6.59
	Fetus	0.33	0.41	2.54	2.35

[a] mmol/min.

[b] AAN = alpha-amino nitrogen.

Table 4—Blood flow of the gravid uterus and fetus in control and heated cows

	Fetal	Total blood flow, liter/min	
Treatment	weight, lb	Uterine	Umbilical
Control	13.2	6.2	1.3
Heated	10.8	4.1	1.0

Table 5—Uptakes of metabolites by the gravid uterus, fetus, and uteroplacenta of control and heat stressed cows[a]

Metabolite	Treatment	Gravid uterus	Fetus	Uteroplacenta
Oxygen	Control	4.37	1.82	4.22
	Heated	3.59	1.42	2.16
Glucose	Control	1.20	0.17	1.52
	Heated	0.66	0.06	0.58
AAN[b]	Control	1.68	1.50	1.97
	Heated	-.22	0.61	-.54

[a] Mmol/min.

[b] AAN = alpha-amino nitrogen.

Table 6—Uterine and umbilical blood flows in Charolais and Hereford cows bearing single or twin fetuses

	Type of	Blood flows, liter/min/fetus	
Cow breed	pregnancy	Uterine	Umbilical
Charolais	Single	7.80	1.24
	Twin	6.34	1.18
Hereford	Single	5.51	1.40
	Twin	4.09	1.06

Table 7—Influence of dam and sire breed on uterine and umbilical blood flows

Dam breed	Sire breed	Blood flows, liter/min		Oxygen uptake, mmol/min	
		Uterine	Umbilical	Uterine	Fetal
Gelbvieh	Charolais	9.64	2.94	7.61	4.25
	Longhorn	7.18	2.22	5.79	3.34
Pinzgauer	Charolais	11.85	3.02	9.83	4.64
	Longhorn	8.34	2.34	7.80	3.66

Table 8—Maternal and fetal influences on blood flow and nutrient uptakes at 230 days of gestation

Breed of cow	Breed of fetus	Blood flow, l/min		Oxygen uptake, mmol/min		Glucose uptake, mmol/min	
		Uterine	Umbilical	Fetus	Uteroplacenta	Fetus	Uteroplacenta
Brahman	Brahman	5.01	2.71	2.32	3.48	.092	.61
	Charolais	4.66	3.65	3.89	1.57	.136	1.43
Charolais	Brahman	7.18	1.88	2.27	2.83	.098	.74
	Charolais	9.24	3.99	3.83	3.93	.215	1.82

Table 9—Maternal and fetal influences on growth of tissues of the gravid uterus

Breed of cow	Breed of fetus	Day of gestation	Weights, lbs			Fetal rate of gain[a]
			Fetus	Uterus	Placentomes	
Brahman	Brahman	232	29.1	9.92	6.34	
		271	56.2	14.48	7.91	0.70
	Charolais	232	52.2	13.51	10.27	
		271	74.7	16.49	10.93	0.58
Charolais	Brahman	232	28.4	12.35	6.28	
		271	67.0	17.86	9.24	0.99
	Charolais	232	49.4	19.11	12.26	
		271	103.4	24.60	14.19	1.38

[a] Rate of gain, lb/day, between 232 and 271 days of gestation.

Feedlot and Carcass Characteristics of Heifers: Effect of Ovariectomy and Ovariectomy with Ovarian Autograft

John M. Klindt and John D. Crouse[1,2]

Introduction

Heifers as beef animals traditionally have been discriminated against in the marketplace. This discrimination is the result of ovarian secretions acting on performance and nutrient partitioning, as well as the possibility of pregnancies. The actions of ovarian secretions can be through their differentiational effects, permanent effects on the development and physiology of the individual, and through activational effects, temporary effects expressed only when the activating agent is present. Expression of heat or estrual activity is an example of an activational effect. The fatter carcasses which heifers generally produce is an example of a differentiational effect. Not only do heifers produce a fatter carcass, they are generally considered to be less efficient in conversion of feed to gain. This is often attributed to the estrous cycles and accompanying behaviors, riding and reduced feed intake. Periodically ovariectomy or spaying has been proposed as a means to prevent heifers from exhibiting estrous cycles (Wilson and Curtis, 1896; Dinusson et al., 1950; Kercher et al., 1960; Horstman et al., 1982; Hamernik et al., 1985). Ovariectomy does certainly stop estrous behavior in heifers. However, it also removes the estrogens and other steroids produced by the ovary. Ovarian steroids, particularly estrogens, are well known to have positive actions on growth. A proposed solution to this problem has been to surgically remove the ovaries and transplant a portion of one ovary to the rumen wall or to simply drop one ovary into the abdominal cavity. This process is referred to as ovarian autotransplantation or autografting. Ovaries removed from their normal connections to the uterus do not support normal estrous cycles but do still produce and secrete many of their steroids.

The objective of this study was to determine whether ovariectomy alone or ovariectomy with ovarian autografting would improve the growth performance or carcass quality of heifers in the feedlot. Ovaries were surgically removed, and one ovary was transplanted to the muscles of the flank in the autograft group. This site was chosen in the hope that retrieval of the ovary for determination of its functional status would be possible at slaughter.

Procedure

Animals: The study used 96 crossbred heifers sired by Simmental bulls and crossbred composite bulls (¼ Red Poll, ¼ Pinzgauer, ¼ Hereford, and ¼ Angus) and dams were crossbreeds of Hereford, Angus, Simmental, and Gelbvieh breeding. Surgeries were performed after weaning, approximately 6 mo of age. Ovariectomies were performed via an incision through the left flank. All surgeries were performed under local anesthesia and the animals were administered a broad spectrum antibiotic at surgery. Surgery groups or treatments were: intact, no surgery; sham-ovariectomy, an incision and manipulation of the ovaries without their removal; ovariectomy, both ovaries were surgically removed; and autograft, both ovaries were surgically removed and one ovary was partially bisected and sutured between the muscles of the flank near the incision site. The transplanted ovary was sutured in place with No. 3 silk and long tails of suture left in order to facilitate its localization at slaughter.

Feeding Trial: The feeding trial was initiated about 1 mo after surgery, when the heifers were about 7 mo of age and 530 lb body weight. Heifers were penned by treatment group; six per pen, four pens per treatment group. Heifers had free access to a corn-corn silage diet. From initiation of the trial until an avg weight of about 750 lb, the diet fed was calculated to contain 1.18 Mcal ME/lb and 12.75% crude protein on a dry matter basis. After reaching approximately 750 lb the diet by calculation contained 1.38 Mcal ME/lb and 10.93% protein on a dry matter basis. Heifers were weighed at 4-wk intervals and feed consumption by pen was determined fortnightly. Heifers, when weighing about 1000 lb, were assigned to slaughter by pen after approximately 215 days on trial.

Blood Samples and Progesterone Measurements: Blood samples were collected from each heifer at each weighing. Serum was harvested and ultimately assayed for progesterone. Progesterone is produced by the corpus luteum, which develops following ovulation and regresses prior to the next ovulation. Temporal concentrations of progesterone in each heifer were plotted against time and ovarian cyclicity was subjectively evaluated. A steady pattern of progesterone over time was interpreted to indicate no estrous cycles were occurring. A cyclical pattern of progesterone over time was interpreted to indicate that the heifer had estrous cycles.

Slaughter: Heifers were slaughtered at the MARC abattoir. All heifers in a pen were slaughtered on the same day. At slaughter the area of the flank containing the transplanted ovary was removed on the kill floor. The ovary was located by palpation and location of the silk suture, and dissected from the surrounding musculature. Ovaries of the intact and sham ovariectomy heifers were also collected. Ovaries were examined and the major or most notable structures were recorded. It was possible for one ovary to have more than one major structure. At slaughter, liveweight and hot carcass weight were recorded. The day following slaughter the carcasses were weighed and evaluated according to the USDA standards for lean color, texture, maturity and firmness, skeletal and overall maturity, dark course banding (heat ring), marbling, and the percentage of kidney, pelvic, and heart fat. Backfat thickness and longissimus area (rib eye area) were measured.

Results

There were no differences among the treatment groups in rate or efficiency of gain (Fig. 1). Also, there were few differences among treatment groups in carcass traits (Table 1). The only differences detected were in measures of maturity. Ovariectomized and ovarian autograft heifers had lower maturity scores than those of either of the intact groups, intact or sham-ovariectomized.

Incidence of ovarian cyclicity in intact, sham-ovariectomy and ovarian-autograft groups was determined from the tem-

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[2]The full report of this work was published in J. Anim. Sci. 68:3481-3487. 1990.

poral nature of peripheral progesterone concentrations (Fig. 2). About 80% of the intact heifers cycled during the trial. Incidence of cyclic behavior was reduced in the sham-ovariectomized heifers to about 60%. Monthly progesterone concentrations fluctuated in a manner suggesting occurrence of estrous cycles in about one-fifth of the ovarian autograft heifers. The progesterone concentrations at slaughter were greater in heifers with ovaries in their normal location, intact and sham-ovariectomy groups, than in the ovarian-autograft group. Significant concentrations of progesterone in the circulation of the autograft heifers indicate that the ovaries did maintain some of their steroid synthetic capabilities in the transplanted site.

Ovaries were examined at slaughter. The most prominent ovarian structures found were corpora lutea and small follicles (Table 2). The intact and sham-ovariectomized heifers tended to have more corpora lutea and small follicles than did the ovarian-autograft heifers. In the ovarian-autograft heifers the most prominent ovarian structures were follicles with thick-luteinized walls, which were classified as luteinized follicles. Luteinized follicles were found in about one-third of the ovarian-autograft heifers. In about one-fifth of the ovarian autograft heifers, the ovaries contained no prominent structures. No ovary was found in 20% of the ovarian-autograft heifers. However, the silk suture used to attach the transplanted ovary to the musculature was found in all autograft heifers, indicating the uncovered ovaries were resorbed.

Discussion

No beneficial effects of ovariectomy or ovariectomy with ovarian autograft were apparent. There were no differences in rate or efficiency of gain, or the major carcass traits of dressing percentage, leanness, and rib eye area. These data indicate that neither ovariectomy or ovariectomy with ovarian autograft offers significant benefits in the production of meat from heifers.

These findings (no benefit as the result of ovariectomy), are in agreement with some more recent reports. The present and more recent reports evaluated the performance of heifers which were ovariectomized soon after weaning and went directly into the feedlot. In contrast, earlier reports examined the response of heifers which were ovariectomized as yearlings and before going to summer grass. Also, this study was with heifers of continental breeding which are later maturing. The earlier studies were with heifers principally of British breeding, primarily Hereford and Angus. The younger age at ovariectomy and slaughter, combined with their being of a breeding that is later maturing, may have contributed to some of the differences between the present results and those reported previously.

The heifers were not fed an energy dense ration which would have increased rates of gain. Considering all available information it appears unlikely that feeding a higher energy ration would have altered the conclusions; regardless of energy density of the ration and rate of gains, ovariectomy and ovarian autograft offer no significant benefits in meat production from heifers.

The only carcass traits influenced by treatment were maturity scores. Maturity scores were lower in ovariectomized and autograft heifers, animals without ovaries or less functional ovaries. The reduced functionality of the ovaries was associated with reduced maturational development, indicating ovarian steroids hasten skeletal and overall maturation.

Ovarian functionality was similar among intact and sham-ovariectomy groups. Ovarian-autograft heifers had reduced ovarian function as indicated by progesterone concentrations in the blood. At slaughter, autograft heifers had fewer corpora lutea and a relatively high incidence of luteinized follicles. One-third of the autograft heifers had luteinized follicles. One-fifth of the autograft heifers had no significant structures on their ovaries, indicating that they were not functional or minimally functional. Thus, over half of the autograft heifers had ovaries that appeared abnormal, i.e., luteinized follicles or no functional structures.

In approximately 20% of the autograft heifers no ovary was found at slaughter. These data indicate not all ovaries transplanted survive or remain functional. The presence or absence of an ovary at slaughter in the autograft heifers was not associated with any differences in growth or carcass characteristics.

This information indicates ovariectomy produced no significant benefits in growth or carcass characteristics of feedlot heifers. Ovariectomy with transplantation of the ovary to a site away from its normal location had no effect on the performance of feedlot heifers. Many autograft heifers were actually ovariectomized. These surgical procedures, ovariectomy or ovariectomy with ovarian autograft, offered no benefit in the management environment employed in the present study.

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Table 1—Least-squares means for slaughter measurements of heifers

	Treatment			
	Intact heifer	Sham-ovariectomized heifer	Ovariectomized heifer	Autografted heifer
Number animals	23	24	24	24
Slaughter wt (lb)	993	990	969	976
Hot carc wt (lb)	632	630	626	628
Fat thickness (in)	.36	.35	.41	.38
Ribeye area (in^2)	12.1	12.1	11.9	11.8
Heat ring (score[c])	6.90	7.02	7.23	7.23
Lean: (score)				
color[d]	6.31	6.59	6.59	6.26
firmness[e]	6.33	6.29	6.21	6.29
texture[f]	6.34	6.25	6.21	6.33
Maturity: (score)				
lean[g]	142.3	139.2	137.4	138.6
skeletal[g]	151.7[a]	151.7[a]	137.5[b]	141.7[b]
overall[g]	147.0[a]	145.5[a]	137.4[b]	140.1[b]
Marbling (score[h])	386.7	399.1	423.7	425.4
K,P,H fat[i] (%)	3.01	2.83	3.01	2.99

[a b] Means within a row with different superscripts are different (less than 5% probability the differences are due to chance).

[c] Eight-point scale; seven = slight two-toning effect, six = small two-toning effect.

[d] Eight-point scale; seven = very light cherry red, six = cherry red.

[e] Eight-point scale; seven = firm, six = moderately firm.

[f] Eight-point scale; seven = fine, six = moderately fine.

[g] 100-199 = A = 9 to 30 mo.

[h] 500 = modest, 400 = small, 300 = slight.

[i] Kidney, pelvic and heart fat.

Table 2—Incidence of major structures on the ovaries of heifers at slaughter[a]

Ovarian structures	Intact heifer	Sham-ovariectomized heifer	Autografted heifer
Corpora lutea	48[b]	77[c]	25[b]
Lutenized follicles	0[b]	0[b]	33[c]
Follicles (>8mm)	35[bc]	15[c]	25[b]
Follicles (<8mm)	9[b]	46[c]	0[b]
No. sig. structures	9	15	20
No ovary	0	0	20

[a] Percentage of the heifers in each group with the observed ovarian structures.

[b c] Means within a row not having common superscripts differ (less than 5% probability the differences are due to chance).

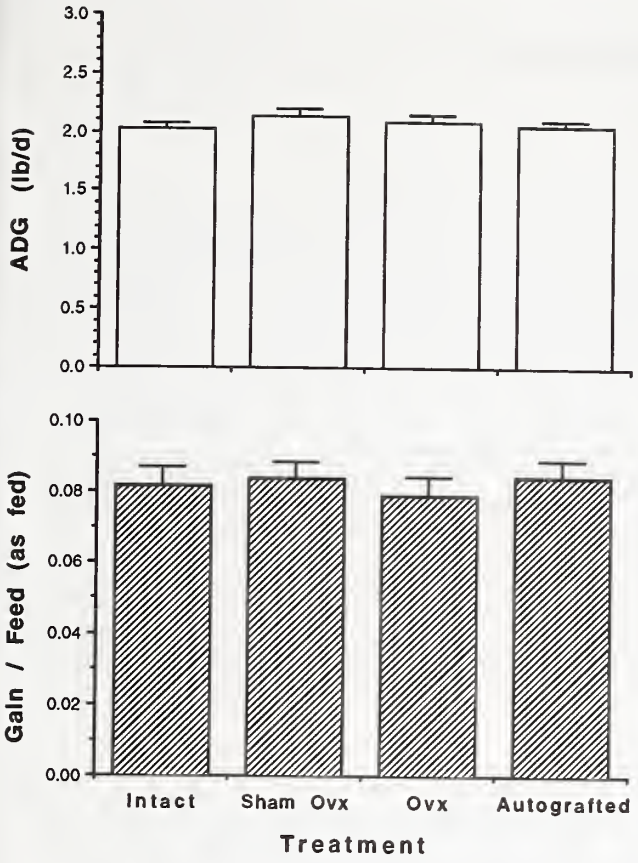

Figure 1 – Avg daily gain (ADG) and efficiency of gain (gain/feed on as fed basis) of heifers of the treatment groups. No significant differences were detected among the treatment groups. Vertical bars represent standard error of the mean.

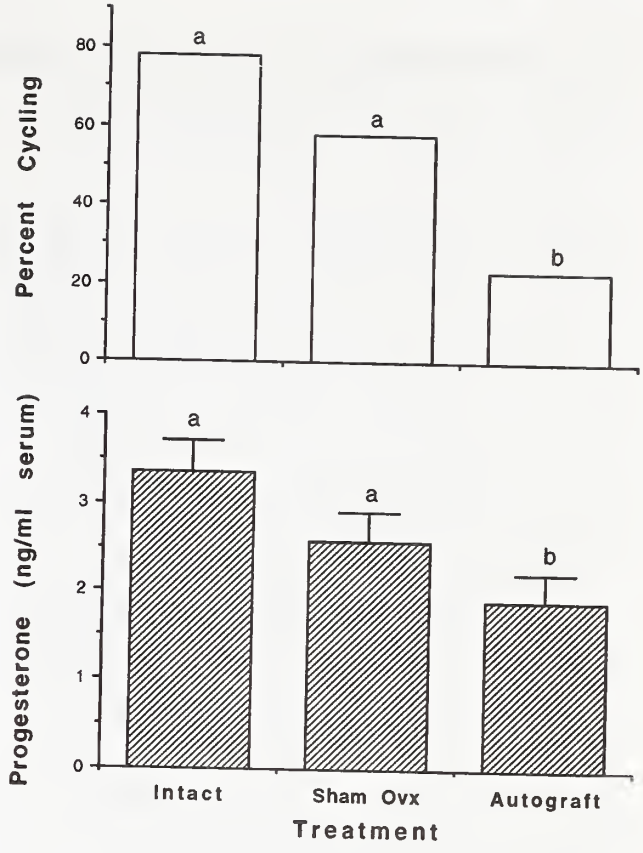

Figure 2 – Incidence of ovarian cyclicity as estimated from temporal progesterone concentrations and mean progesterone in heifers of groups which had ovaries at slaughter.
[a b] Bars with different letters differ (5% probability that differences are due to chance).

Is Fiber Digestion in the Rumen Reduced by Catabolite Repression?

Kevin L. Anderson and Vincent H. Varel[1]

Introduction

Bacteria which degrade cellulose play a key role in animal digestion of plant material. As with all bacteria, these cellulolytic bacteria are able to regulate their growth and enzymatic activity by a number of mechanisms. One of these regulatory mechanisms may be catabolite repression.

This repression refers to the ability of certain bacteria to stop the metabolism of one substrate in preference to a second substrate. Sometimes termed "glucose effect," this repression is observed, for example, as the bacterium *Escherichia coli* grows on lactose. When glucose is then added to the growth medium, *E. coli* will stop utilizing lactose and use glucose instead. Thus, glucose causes a repression of lactose utilization.

Since soluble carbohydrates may be present in the microecosystem of cellulolytic bacteria, especially near the site of plant degradation, there is a potential for catabolite repression. This would negatively affect bacterial cellulolytic activity, thereby reducing efficiency of ruminal plant digestion. However, it is not clear which, if any, cellulolytic bacteria are capable of catabolite repression. Previous studies have not been conclusive because of a variety of confounding factors resulting from the experimental design. These include difficulty of adequately measuring substrate depletion, metabolic effect of other regulatory systems, and inhibition of cellulolytic activity by a decreasing pH of the growth medium.

Glucose analogs are chemical compounds which have the ability to "trick" the bacterial cell into thinking it is a food substrate, when in fact the analog is an imitation compound which the cell cannot metabolize for growth. Therefore, a small concentration of an analog can simulate the regulatory effect of glucose without serving as a growth substrate or interfering with carbohydrate transport. Since energy metabolism and bacterial growth will result only from cellulose degradation, many confounding factors are eliminated.

Procedure

Several strains of ruminal and nonruminal anaerobic (growth without oxygen) cellulolytic bacteria were studied (see Table 1). All strains were grown in a basal medium containing 20% incubated rumen fluid. As the sole carbohydrate source, 0.5% glucose, fructose, xylose, maltose, or 1% balled-milled cellulose was added to the medium.

All bacterial strains were grown for at least 12 generations on a noncellulose carbohydrate substrate prior to inoculation of cellulose medium. When a strain could grow on a sugar other than glucose, this compound was used as an inducing substrate (see Table 1).

Each strain was then inoculated into six bottles of the basal medium (30 ml) containing 1% cellulose. As a treatment group, the analog methyl-glucose was then added to three of these bottles. All bottles were incubated at 102°F while being continually agitated.

Periodically 1.5 ml was removed from each bottle, filtered, and analyzed for fermentation products. Since growth resulted only from cellulose degradation, the rate of production of these fermentation products was used as an indicator of metabolic activity. Concentrations of this metabolite from the treatment group were compared to those of the respective control group. Only treatment groups showing almost complete repression of metabolic activity for at least a 24 h period were identified as catabolite repression.

Results

Eight species of anaerobic cellulolytic bacteria were studied for their ability for catabolite repression. Under the experimental conditions used, only the rumen strain *C. longisporum* OC4 was found to exhibit catabolite repression. This repression was most pronounced when OC4 was grown on the sugar fructose (see Figure 1), apparently because this required the cells to induce the maximum number of cellulolytic genes while in the presence of the glucose analog. Although *C. longisporum* OC4 is a ruminal isolate, it is not routinely found in the rumen. Its susceptibility to catabolite repression offers one explanation why it does not appear to be a common cellulolytic bacterium of the rumen.

This study suggests that catabolite repression is not a significant factor in cellulolytic degradation. In fact, several of the strains studied grew better on cellulose than on soluble carbohydrates. This suggests that cellulose was preferred over other substrates, even glucose. Furthermore, *Clostridium* sp. 54408 and *C. polysaccharolyticum* B do not use glucose as a substrate. Therefore, their lack of response to the glucose analog was predictable.

[1]Anderson is a research associate, and Varel is a research microbiologist, both in the Nutrition Research Unit, MARC.

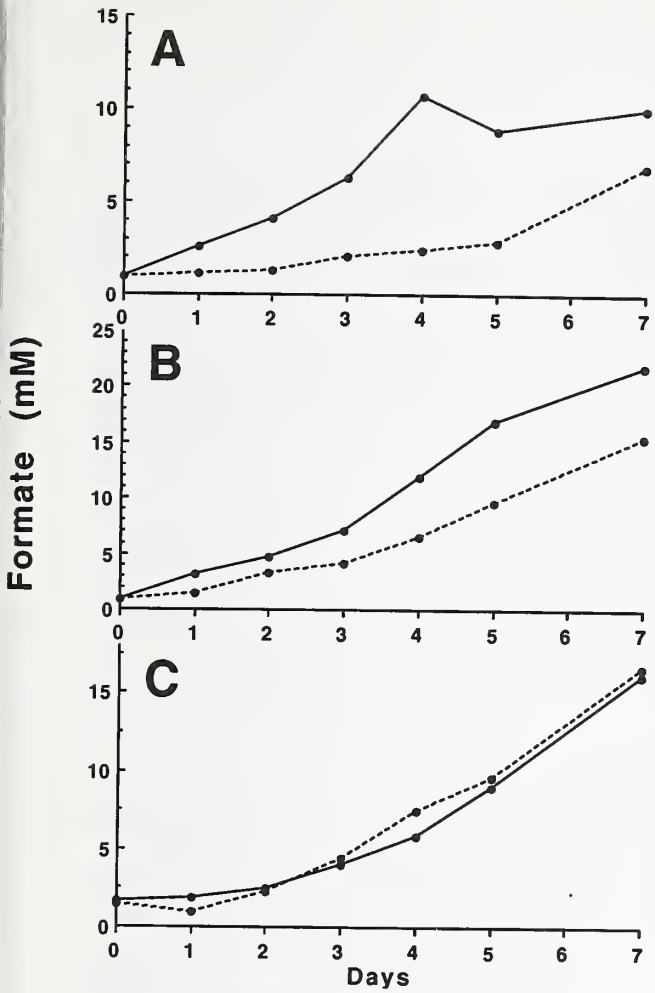

Figure 1 – Formate production by *Clostridium longisporum* OC4 grown on cellulose (_____) and cellulose + 2 mM methyl glucose (------). Cells were grown at least 12 generations on a) fructose, b) glucose, c) cellobiose before inoculation into cellulose medium.

Table 1—Cellulolytic bacterial strains studied. Cells were fully induced (12 generations) on respective substrates prior to inoculation into cellulose medium. Growth on cellulose was determined by measuring the increase of the respective metabolite

Bacterium	Substrate	Metabolite	Repression[a]
Costridium cellulolyticum H10	fructose	acetate	-
Clostridium cellobioparum 3359	glucose	acetate	-
Clostridium polysaccharolyticum B	xylose	formate	-
Clostridium longisporum OC4	fructose	formate	+
Clostridium sp. 54408	maltose	butyrate	-
Eubacterium cellulosolvens 5494	fructose	butyrate	-
Ruminococcus albus 7	fructose	acetate	-
Fibrobacter succinogenes S85	glucose	succinate	-

[a] Detection of catabolite repression: (-) not detected, (+) detected.

Omasal and Duodenal Nutrient Flow in Steers.

Kelly K. Kreikemeier, Gary P. Rupp, and Louis J. Perino[1]

Introduction

Feedstuffs are degraded in the rumen, providing energy and nutrients for microbial growth. Volatile fatty acids produced during this process are absorbed and used as an energy source by the animal. The bacteria that are produced and unfermented feed residue flow out of the rumen to the small intestine where further digestion and absorption occurs. Of the total protein flowing to the small intestine, 50 to 90% is microbial protein. It is of high quality, well digested and well used by the animal.

Currently, estimates of the amount of microbial protein synthesized in the rumen vary considerably. We do not understand what portion of this variation can be attributed to nutritional factors such as feed intake, forage versus grain, animal differences, etc. Because the amino acid profile of dietary ingredients and ruminal microflora differ, knowing what influences the amount and proportion of each flowing to the lower gut is very important if we are going to extend our understanding of protein utilization in cattle.

The amount of microbial protein flowing to the small intestine in cattle is measured in the following manner: 1) cattle are surgically fitted with a ruminal and duodenal cannula for digesta sampling; 2) After a dietary adjustment period, ruminal bacteria are harvested and duodenal (anterior small intestine) digesta is sampled; 3) Laboratory analyses are conducted and the amount of microbial protein flowing at the duodenum is determined. Dietary protein flow at the small intestine is calculated as the difference between total protein flow and microbial protein flow.

This approach has been used by researchers for several years, but it may have limitations. First, it is unable to account for endogenous nitrogen flowing at the duodenum, due to either sloughed cells or abomasal secretions. Any endogenous nitrogen would overestimate the flow of dietary protein. Secondly, it is assumed that a sample of bacteria obtained from the rumen represents bacteria flowing out of the rumen. If this assumption is not correct, then our estimate on the amount of microbial protein flowing at the duodenum is not correct.

The potential limitations in research techniques might account for the large variation in the amount of microbial protein synthesized. These limitations might be overcome if a different cannulation technique were employed so we could sample digesta flowing out of the rumen. To our knowledge, this approach has been used in sheep by four different investigators with limited success. Building on the reported limitations of these efforts in sheep, we wanted to conduct a similar surgical preparation in cattle. We had two objectives, 1) determine if nitrogen flowing out of the rumen differed from nitrogen flow at the duodenum, and 2) determine if the composition of bacteria in the rumen differed from bacteria flowing out of the rumen.

Procedures

Six steers (649 pounds) were surgically fitted with digesta sampling cannulae in the rumen omaso-abomasal orifice, abomasum, and duodenum. A flexible nylon sleeve, located in the abomasum and attached to the omasal cannula, was exteriorized via the abomasal cannula during digesta collection. The location of cannulas allowed us to collect digesta in the rumen, digesta flowing out of the rumen, and digesta flowing into the small intestine.

Based on general appearance, body temperature, and feed consumption, steers recovered from surgery in three or four days. At this time, they were transported to the metabolism barn and placed in stalls (3.5 ft by 7 ft) containing rubber floor mats. Steers were turned outside in a dirt lot for exercise 2 hr per day at least three days per week. Steers were fed with automatic feeders so that they were offered a portion of feed every 2 hours.

Three experiments were conducted (Table 1): 1) 95% concentrate fed at maintenance (2760 g organic matter (OM)/day), 2) 95% concentrate fed ad libitum (3484 g OM/day), and 3) low quality brome hay based diet fed ad libitum (2927 g OM/day). For each experiment, steers were fed the diet at least 14 days. Omasal and duodenal digesta were then collected (200 ml) three times daily for three consecutive days. Ruminal digesta was also collected. Bacteria were harvested from ruminal and omasal digesta and laboratory analyses were conducted.

Results and Discussion

Organic matter flow (Table 2) at the omasum was similar to organic matter flow at the duodenum in Experiments 1 and 2 and slightly higher in Experiment 3. Statistically these values were not different. Fluid flow was 29 to 40 lb greater at the duodenum than at the omasum. Our duodenal cannula was 4 to 6 inch distal to the pylorus and our sampling technique assured that no digesta backflow nor pancreatic secretions were collected. Therefore, the greater fluid flowing at the duodenum probably originated from abomasal fluid secretions.

Total volatile fatty acid flow (VFA) was much greater at the omasum than at the duodenum, indicating a significant absorption of VFA across the abomasum. The amount absorbed across the abomasum varied from 370 to 570 mmol/day and this represents approximately 5% of ruminal VFA production. Volatile fatty acid concentration was very low (about 5 mM) at the duodenum, which is consistent with reports that almost all (greater than 95%) of the VFA produced ruminally are absorbed before reaching the duodenum.

Purine flow at the duodenum was higher than purine flow at the omasum in Experiments 1 and 3, but lower in Experiment 2. Purines are commonly used as a microbial marker to measure microbial protein flow to the small intestine. If purine flow at the duodenum had been consistently higher than purine flow at the omasum, then microbial protein flow would have been overestimated.

Nitrogen flow at the duodenum was very similar to nitrogen flow at the omasum in Experiments 1 and 2, and only slightly higher in Experiment 3. Some earlier research suggested that the abomasal mucosa secretes nitrogen, which would contribute to duodenal nitrogen flow. If this occurred to any measurable extent, investigators using duodenally cannulated cattle would overestimate dietary protein escaping ruminal fermentation. Alpha-amino, urea, and ammonia nitrogen are soluble nitrogen components. Tallied, they accounted for less than 5% of the total nitrogen, and their flow was similar at the omasum and duodenum.

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In order to determine the amount of bacterial protein in duodenal digesta, a representative sample of ruminal bacteria must be obtained. Their marker to nitrogen ration is then used to calculate the proportion of duodenal nitrogen that is of microbial origin. We harvested bacteria from ruminal digesta and from omasal digesta to determine if their marker to nitrogen ratio differed (Table 3). In all three experiments, omasal digesta contained three to five times more purines than ruminal digesta. This was likely due to a greater enrichment of bacteria in omasal digesta. The complete diet contained 0.10% purines; whereas, the bacteria contained 5 to 7% purines.

The amount of bacteria harvested per unit weight of digesta was higher from omasal digesta, and this was likely due to the greater bacterial enrichment of omasal digesta. Bacteria harvested from omasal digesta contained more nitrogen and purines than bacteria harvested from ruminal digesta. Despite their changing composition, the nitrogen to purine ratio was similar between bacteria harvested from ruminal digesta and bacteria from omasal digesta. Because their nitrogen to purine ratio was similar, the calculated proportion of duodenal protein flow attributed to bacterial protein would not be affected.

Lower nitrogen and purine concentration in ruminally harvested bacteria has other implications with research techniques and data interpretation. The difference between the feed organic matter consumed and digesta organic matter flowing at the duodenum is the amount of organic matter that apparently disappeared in the rumen. Because duodenal digesta contains both undigested feed residue and bacteria, correcting for the bacterial component is required to calculate true ruminal organic matter disappearance. In these experiments, calculating "apparent" versus "true" ruminal organic matter disappearance would be affected differently using data from ruminal or omasal bacteria. Calculations for efficiency of microbial protein synthesis would be affected as well.

In conclusion, 1) there was a net appearance of fluid and disappearance of volatile fatty acids across the abomasum in steers, 2) the technique of fitting cattle with ruminal and duodenal cannulas to measure the amount of feed protein and microbial protein flowing at the duodenum is not confounded by abomasal nitrogen or purine secretions, and 3) composition of bacteria flowing out of the rumen differs from bacteria in the rumen.

Table 1—Diet composition[a]

Ingredient	Percentage of diet dry matter	
	Exp. 1 and 2	Exp. 3
Rolled corn	85.69	0
Cane molasses	5.00	5.0
Brome hay	5.00	92.11
Limestone	1.47	.96
Urea	1.39	1.07
Dicalcium phosphate	.40	.11
Salt	.30	.30
Potassium chloride	.21	0
Sulfur	.09	.02
Vitamin ADE premix[b]	.05	.05
Mineral oil	.05	.05
Trace mineral premix[c]	.05	.05
Magnesium oxide	.03	0
Rumensin-60[d]	.02	.02
Cromic oxide	.25	.25

[a] Diet formulated to contain 13% protein, .7% calcium, .2% magnesium, .35% phosphorus, .7% potassium, .21% sulfur. Actual protein in Exp. 3 was 9.5% because the brome hay contained only 7.0% crude protein.
[b] Contains 8,800,000 IU Vitamin A, 880,000 IU Vitamin D, and 880 IU Vitamin E per kg of premix.
[c] Contains 14% calcium, 12% zinc, 8% manganese, 10% iron, 1.5% copper, .2% iodine, and .1% cobalt.
[d] Added so the diet contained 25 ppm monensin.

Table 2—Effect of digesta sampling site on nutrient flow

Item	Exp. 1		Exp. 2		Exp. 3	
	Omasum	Duodenum	Omasum	Duodenum	Omasum	Duodenum
Organic matter flow, lb/d	1.67	1.53	3.33	3.21	2.83	3.42
Fluid flow, lb/d	34.1	65.3	71.5	111.1	84.7	113.9
VFA flow, mmol/d	589	212	932	359	1158	695
Purine flow, lb/d	.038	.050	.073	.068	.032	.037
Nitrogen flow, lb/d	.089	.089	.155	.157	.087	.097
a-amino-nitrogen flow, lb/d	.002	.003	.002	.002	.002	.002
Urea-nitrogen flow, lb/d	.001	.001	.001	0	.001	.001
Ammonia-nitrogen flow,lb/d	.001	.001	.001	.001	.001	.001

Table 3—Effect of sampling site on the composition of harvested bacteria

Item	Exp. 1		Exp. 2		Exp. 3	
	Rumen	Omasum	Rumen	Omasum	Rumen	Omasum
Digesta purine, %	0.82	2.38	1.01	2.62	0.28	1.16
Bacterial OM harvested, %	6.7	14.9	5.4	7.9	1.88	4.38
Bacterial nitrogen, %	7.31	8.56	6.73	8.43	5.76	7.33
Bacterial purine, %	4.93	6.75	4.73	5.64	3.23	4.49
Nitrogen/purine	1.56	1.29	1.58	1.65	1.87	1.66

Fiber Degrading Microorganisms from Bison, Cattle-Bison Hybrids and Cattle.

Vincent H. Varel and Burk A. Dehority[1]

Introduction

The limiting factor in forage plants which prevents more complete degradation by ruminants is the fiber component. Sometimes called lignocellulose, this fraction is primarily composed of cellulose, hemicellulose, and lignin. Forages are normally high in lignocellulose when compared to cereal grains, therefore they are lower in digestibility, which in turn results in reduced efficiency of animal production. Studies have shown that a small increase in forage digestibility, such as 7 to 12%, can result in increases of 30 to 40% in animal gain. These numbers provide a substantial incentive to further study the factors which limit the degradation of forage with the overall objective of enhancing the efficiency of animal production.

Enzymes produced by microorganisms are solely responsible for degrading forage fiber. The digestive enzymes produced by the animal do not break down fiber. Therefore, it is essential to understand the microorganisms and their interactions, primarily those which occur in the rumen, to improve the degradation process of forage. Approximately 80% of the animal energy is obtained from volatile fatty acids produced by microorganisms in the rumen.

Various studies suggest that the North American buffalo (*Bison bison*), has a superior ability, when compared with domestic cattle (*Bos taurus*), to digest low quality forages. Digestion coefficients of all nutrients, including dry matter, crude protein and fiber have been shown to be greater in bison than in Hereford steers. Bison appear to show superior digestibility when poor quality, low protein diets are fed. Explanations offered for the superior digestion coefficients were that a greater recycling of nitrogen to the rumen and a reduced rate of digesta passage occur. Previous studies have not compared the cellulolytic or protozoal populations between bison or domestic cattle. The objective of our studies was to compare cellulose degrading microorganisms and ruminal fermentation parameters between bison, cattle-bison hybrids, and cattle, fed three levels of low quality alfalfa.

Procedure

Five steers, 450 to 550 lb, from each of three groups, bison (*Bison bison*), cattle-bison hybrids (1/2 bison X 1/2 cattle, breed Charolais), and crossbred cattle (*Bos taurus*), were fed three levels of alfalfa hay (crude protein 13%); 100% ground alfalfa, 75% alfalfa - 25% corn, and 50% alfalfa - 50% corn. Animals were penned by species and fed individually ad libitum with electronic headgates. Each diet was fed for 12 weeks. During this time samples of ruminal fluid were obtained from each animal and analyzed for cellulolytic and protozoal populations, pH, ammonia, and volatile fatty acids.

Results

The total numbers of viable or cellulolytic bacteria were not different among animal groups fed diets containing three proportions of alfalfa. Total viable bacteria ranged from 2.16 X 10^9 to 5.44 X 10^9/ml of ruminal fluid, and the cellulolytic population represented 1.2 to 3.6% of the total viable bacteria.

The four major groups of cellulose degrading bacteria were observed in all animals and diets as indicated in Table 1. When a 100% alfalfa diet was fed, a greater percentage of *Fibrobacter succinogenes* isolates were obtained from bison (58%) than from hybrids (36%) or cattle (33%). These isolates are well known for their ability to degrade crystalline cellulose, which is considered to be the more difficult fraction of fiber to degrade, when compared to amorphous cellulose which *Ruminococcus albus* degrades most efficiently. *R. flavefaciens* degrades both types of cellulose, while *Butyrivibrio* does not degrade either type of cellulose very well. Assuming that *F. succinogenes* is a more efficient degrader of crystalline cellulose as many studies have demonstrated, the higher percentage of these organisms in bison may mean higher fiber digestion in these animals.

When the 100% alfalfa diet was fed, *Butyrivibrio* represented only 7% of the cellulose degrading isolates from the bison, which is significantly lower than the 29% for hybrids and 13% for cattle. Besides degrading cellulose this organism also degrades starch. Therefore, the numbers of *Butyrivibrio* increased as the amount of corn was increased in the diet, i.e., 7, 15, 39%; 29, 30, 50%; 13, 48, 57% respectively, for the bison, hybrids, and cattle, when corn was fed at 0, 25 or 50%. Neither group, *R. albus* or *R. flavefaciens*, demonstrated a distinctive trend in the animal groups or diets which might suggest that they are responsible for greater fiber degradation.

Except in one bison, cellulose degrading clostridia were not found as predominant organisms. Earlier studies have shown that clostridia found in the rumen are the most active in degrading forage plant cellulose. Work from the clostridial isolate in our study, *Clostridium longisporum*, confirmed this. The bison isolate degraded 5% more of the total forage fiber (cell walls) and 15% more of the hemicellulose component of the fiber when it was compared to other pure culture isolates from the rumen which degrade cellulose (Fig. 1). It is possible that our techniques were not adequate to isolate more clostridia which degrade cellulose or these strains are present in the rumen only sporadically. Indian water buffalo have populations of cellulose degrading clostridia as high as 4% of the total microbial population. Water buffalo (*Bos bubalis*) would normally be expected to receive a poor quality diet compared to our *Bos taurus* cattle; thus, the cellulolytic clostridia may have been ecologically selected by the buffalo to digest these poor quality low protein diets. Simple studies such as cross inoculation of *Bos taurus* cattle with rumen contents of water buffalo should be conducted to determine if forage fiber digestion can be increased in *Bos taurus* cattle.

Less is known about the fiber degrading ability of protozoa when compared to bacteria. In our study, for bison, total protozoa varied in individual animals from 2.08 X 10^4/ml when they were fed 100% alfalfa to 76.16 X 10^4/ml with 50% alfalfa - 50% corn. This compares favorably to other studies on bison. No unusual species of protozoa were observed in bison and they were similar to those that occur in domestic ruminants. Generic distribution of protozoa varied in comparison with hybrids and cattle.

No differences in ruminal pH, volatile fatty acids or ammonia nitrogen concentrations among the animal groups

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were observed when 100% alfalfa was fed. However, ruminal pH was lower for bison than for the hybrids and cattle when 25 or 50% corn was added to the diet. Ammonia nitrogen concentrations and volatile fatty acids were higher for the bison than for the hybrids or cattle when 50% corn was fed.

In summary, our studies indicate that there are differences in the percentages of cellulose degrading bacteria and protozoa inhabiting the bison rumen compared with those of cattle. Also when a mixture of 50:50 alfalfa-corn was fed, ruminal pH, volatile fatty acids, and ammonia nitrogen were different between bison and cattle, supporting different microbiological or animal nutritional responses to this diet. Growth efficiency data with these animals are currently being analyzed and should assist us in the interpretation of our data and indicate whether fiber was utilized more efficiently by bison in this study.

Recently the fiber degrading enzymes from *F. succinogenes* of an Asian water buffalo have been compared to those of a *F. succinogenes* strain from domestic cattle. The three enzyme activities assayed from the buffalo strain were all greater than those of the cattle strain. This is unique because *F. succinogenes* is one of the major cellulose and hemicellulose degrading microorganisms in the bovine rumen and our results from this study indicate that *F. succinogenes* was the predominant isolate found in the bison on the high forage diet.

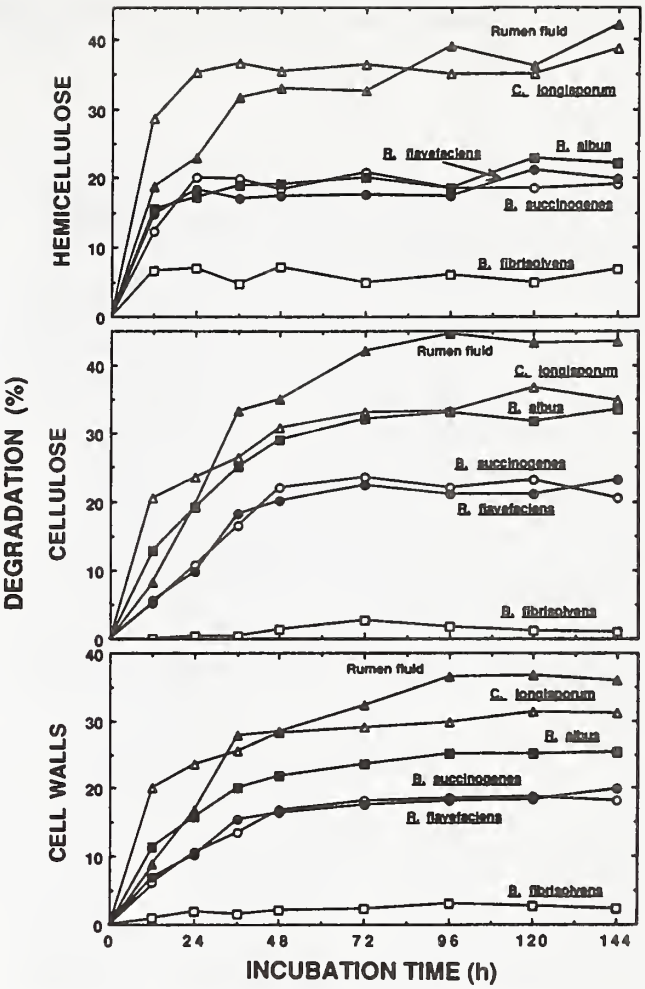

Figure 1 – Comparison of *in vitro* degradation of alfalfa fractions by strain B6405 (*Clostridium longisporum*), mixed culture rumen fluid, and other ruminal celluloytic strains. □, *Butyrivibro fibrisolvens*; ●, *Ruminococcus flavefaciens*; ○, *Bacteriodes succinogenes*; ■, *R. albus*; △, *C. longisporium*; ▲, *rumen fluid.*

Table 1—Percentage of ruminal cellulose degrading bacteria from bison, cattle-bison hybrids, and cattle fed diets containing three levels of alfalfa[a]

	Percent of ruminal cellulolytic bacteria that were:[a]														
	Butyrivibrio			R. albus			R. flavefaciens			F. succinogenes			Unknown[b]		
Species	100	75:25	50:50	100	75:25	50:50	100	75:25	50:50	100	75:25	50:50	100	75:25	50:50
Bison	7	15	39	29	29	17	.8	7	17	58	36	16	4	12	11
Hybrids	29	30	50	32	36	13	1.0	9	5	36	12	26	2.4	12	5
Cattle	13	48	57	43	10	6	2.6	7	3	33	18	18	8	17	16

[a] 100 = 100% alfalfa; 75:25 = 75% alfalfa, 25% corn; 50:50 = 50% alfalfa, 50% corn.
[b] Not identified or culture was lost before it could be characterized.

Energy Expenditures of Mature Cows During the Production Cycle

Calvin L. Ferrell and Tom G. Jenkins[1]

Introduction

The cow uses about 65% of the feed energy used in the production of beef cattle. Of that, about 74% is used for maintenance of the maternal body, 18% for lactation and 8% for pregnancy in the mature, producing beef cow. The growing-finishing animal uses about 35% of the total feed energy used for beef production. In the growing-finishing animal, maintenance costs may vary from 30 to 100% of the total feed energy consumed, with the lower proportion being at high intakes and rates of gain. Typically, in feedlot cattle this value ranges from 30 to 40%, whereas in cattle growing more slowly, such as stocker cattle, maintenance costs represent about 50 to 70% of the total. As a result, energy expenditures for maintenance are relevant to all phases of beef production, but are generally of greater importance in the cow herd.

Numerous breeds of cattle are currently available to beef cattle producers. Large differences among breeds for important traits such as mature size, growth rate, body composition, and milk production have been well documented. To effect improvements in efficiency, both required feed input and product output need critical examination. Differences among breeds or genotypes have also been observed with regard to energy requirements and/or efficiency of energy utilization for maintenance and weight or energy gain. Some of the research efforts at MARC in this regard were summarized in the previous Beef Research Progress Report (Ferrell and Jenkins, 1988). Those studies indicated that, in general, there appears to be a positive association between genetic potential for productivity and maintenance requirements. Stated another way, there is an antagonistic relationship between potential productivity and feed requirements for maintenance. Further understanding of relationships between utilization of feed energy and productive potential is needed in order to select appropriate genotypes for given production environments. The objective of the present study was to evaluate the relationship of animal energy expenditures to feed available in diverse genotypes.

Procedures

In 1986, mature, multiparous Hereford (10) and Simmental (10) cows were assigned to the study. Within each breed, cows were assigned to four levels of feed intake and were fed, individually in dry lot, at those levels for four years. Hereford cows received 47, 60, 72 and 85 grams dry feed per metabolic body size (MBS, weight in kilograms raised to the 0.75 power) and Simmental cows received 60, 72, 85, and 98 grams dry feed per MBS daily. Feed allowances were increased during lactation to sustain maternal weight. The feed used consisted of 70% ground alfalfa hay and 30% rolled corn and was supplemented with minerals and vitamins A, D and E. Cows were weighed at 28-day intervals. Milk production was measured at 28-day intervals, beginning 14 days after parturition.

Heat production was measured by open circuit, indirect calorimetry in this study. For this measurement, oxygen consumption and carbon dioxide and methane production are determined and heat production is calculated from those determinations by established procedures. Measurements were recorded for cows five times each year. Measurements were thus made on cows that were in four different physiological states: pregnant-nonlactating, nonpregnant-lactating, pregnant-lactating, and nonpregnant-nonlactating. Cows failing to conceive during the breeding season were measured to provide data on nonpregnant-nonlactating cows. Prior to the initiation of the study, all cows were trained to the facilities and equipment. During the acclimatization and collection periods, cows had access to water and daily feed allowances were provided. During lactation, calves had access to the cows.

Results and Discussion

Of the feed energy consumed, part is retained as body tissue, part is deposited in the fetus and other tissues of the uterus during pregnancy, part is secreted in milk during lactation and the remainder is lost as heat. Thus, heat production relative to feed intake is a measure of the inefficiency of the animal. In this study, each cow was fed a constant daily amount throughout the study (except for adjustments during lactation). As a result, weights plateaued, thus cows were at weight stasis which is essentially equivalent to maintenance. Weights maintained differed, depending on feed intake and efficiency of feed utilization.

The ranking of Simmental cows relative to Hereford cows was similar regardless of physiological state. Pooled over physiological state, estimated heat production of Hereford cows was less than estimated for Simmental cows at zero feed intake (Fig. 1). However, heat production increased more rapidly as feed intake increased in Hereford than in Simmental cows. The relationships of heat production to feed intake of the Hereford and Simmental cows intersected at 193 gram feed dry matter per kilogram body weight. Above that point, Simmental cows produced less heat than Hereford cows.

We suggest these results indicate the Hereford cows were more adaptable to low feed intakes than were the Simmental cows. Conversely, Simmental cows apparently used feed energy more efficiently than Hereford cows when chronically adapted to high levels of feed intake. These results are consistent with the concept that if environmental conditions limit productivity (e.g., inadequate nutrition), genotypes having lower production potential, and associated lower maintenance, are less adversely affected than genotypes having higher genetic potential. Conversely, if environmental conditions are not limiting, genotypes having the greatest potential productivity are favored.

FIGURE 1. RELATIONSHIP BETWEEN DAILY HEAT PRODUCTION AND DRY MATTER INTAKE POOLED ACROSS PHYSIOLOGICAL STATES.

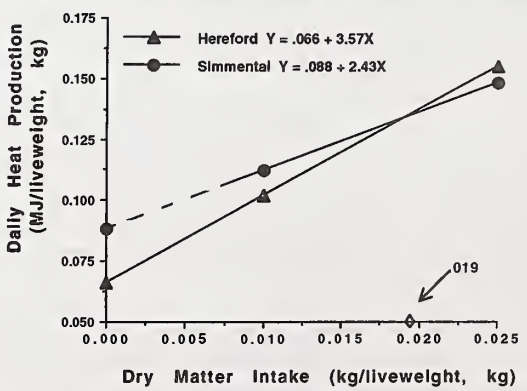

Figure 1 – Relationship Between Daily Heat Production and Dry Matter Intake Pooled Across Physiological States

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Improving the Microbiological Quality of Meat

James S. Dickson and Gregory R. Siragusa[1,2]

Introduction

Microbial contamination of animal carcasses is a result of the necessary procedures required to process live animals into retail meat. The contamination can be minimized by good manufacturing processes, but the total elimination of bacteria of public health significance is difficult, if not impossible. A variety of methods have been developed to improve the microbiological quality of meat, although most of the current methods focus on washing and sanitizing the carcasses, prior to chilling.

The beef slaughter process begins by humanely stunning the animal, bleeding, and then removing the hooves and head. The hide is removed, and the carcass is eviscerated and split into halves. The carcass halves are washed and then cooled to refrigeration temperatures. The initial research on carcass washing was with washing the split carcass which, as the final step before chilling, was intended to remove as much of the total physical and microbiological contamination as possible. Manual washing was refined with equipment that automatically washed the carcasses. The automated systems were more consistent in operation than a manual system, and also reduced the amount of water used in washing. A further refinement of the automated systems was the inclusion of a sanitizing rinse immediately after washing. The sanitizing rinse uses food grade antibacterial compounds to inhibit the growth of any bacteria remaining after the initial wash. The sanitizers typically are organic acids, such as acetic (vinegar) or lactic acid (naturally occurring in cheese).

The automated washing and sanitizing systems were successful in improving the microbiological quality of beef carcasses. However, since much of the contamination of the surface of the carcass occurs during the hide removal, a second washing station was inserted immediately after hide removal and prior to evisceration (termed "pre-evisceration" washing). The process of pre-evisceration has been patented by a major U.S. meat packer.

The traditional method of cooling carcasses was by forced air refrigeration. In the 1970's, a new cooling process was developed which misted cold water on the carcasses in conjunction with refrigeration. This new process increased the cooling rate by evaporative cooling, and reduced the weight loss of the carcass which normally occurred during traditional chilling. The process used chlorinated water to inhibit bacterial growth, and was patented as "chlor-chil." Since that time, other sanitizers have been incorporated into the spray water on an experimental basis.

Although there were some data in the scientific literature on each of these processes individually, we wanted to evaluate the entire system under controlled conditions. Therefore, our objectives were to determine the effectiveness of pre-evisceration and post-evisceration washing and sanitizing, followed by spray chilling, in reducing the population of salmonellae on beef. This research was conducted in the laboratory, as a feasibility study to establish processing guidelines for full-scale equipment currently being installed in the abattoir at the MARC.

Procedure

Bacterium. A strain of *Salmonella california*, naturally resistant to a potent antibacterial compound (naladixic acid), was grown and maintained in tryptic soy broth, a general bacterial growth medium. The marker bacterium was suspended in manure collected from cattle fed a corn silage diet prior to use, to simulate a "worst case" contamination situation. The marker bacterium was easily differentiated from the other bacteria in the manure by its ability to grow on selective culture media containing naladixic acid.

Tissue. Postrigor beef tissue was obtained as boneless trim from the abattoir at the MARC. The tissue was separated into lean and adipose tissues, sliced into 0.2 in thick slices, sterilized with gamma radiation, and stored frozen until use. Prior to use, the slices were cut into squares and tempered to room temperature. Tissue produced in this manner had previously been determined to be representative of prerigor tissue, in terms of numbers of bacteria which would attach and the sensitivity of the attached bacteria to organic acids.

Experimental design. The tissue samples were inoculated by immersing them in the manure containing the marker strain of salmonella for 5 min. After inoculation, the tissue was washed and sanitized (pre-evisceration), allowed to stand for 10 min to simulate the normal delay in processing, washed and sanitized a second time (post-evisceration), and then spray chilled. Washing and sanitizing treatments were simulated by vigorously washing the tissue in distilled water (washing) or 2% acetic acid (sanitizing). Because previous research had indicated an enhanced sanitizing effect if the acid was warmer than room temperature, the acid was applied at 131°F. Spray chilling was simulated by briefly dipping the tissue in 41°F water at 30 min intervals for 4 hours.

Enumeration of bacteria. The samples were homogenized and bacterial populations were enumerated on a variety of culture media. These media included a nonselective, general growth medium (TSA) and two media which were specifically intended to isolate salmonella (EF-18 and MAC). Naladixic acid was added to all three media to specifically select for the marker bacterium. Use of both the nonselective and selective media allowed the potential differentiation between normal, healthy bacterial cells and those which may have been injured by the sanitizing process.

Results

A single washing treatment, comparable to a final post-evisceration wash, removed approximately 90-95% of the contaminating bacteria from lean and adipose tissue, when used in combination with spray chilling. The inoculation process left the tissue samples covered with manure, and the reduction in bacterial numbers was due primarily to the removal of gross physical contamination. This emphasizes the importance of carcass washing, since much of the initial bacterial population can be removed by adequate washing. The combination of pre- and post-evisceration washing and sanitizing reduced the population by another 90% on lean tissue and by greater than 99% on adipose tissue. If the ini-

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[1] Dickson is a research food technologist, Meats Research Unit, MARC, and Siragusa is a microbiologist, Meats Research Unit, MARC.

[2] The full report of this work was published in J. Food Prot. 54:514-518, 1991, and J. Food Sci. 56:191-193, 1991.

tial population of salmonellae were 1000 cells per square inch, these processes in combination would reduce the population to approximately ten cells per square inch on lean and one cell per square inch on adipose tissue. These processes would be expected to result in comparable reductions in the populations of other bacteria, including both spoilage and bacteria of public health significance.

Bacteria on adipose tissue tend to be more susceptible to organic acid sanitizers than those on lean tissue, as this report and other research have demonstrated. This effect has been attributed to the difference in the microenvironments on the surface of these two tissue. Bacteria apparently attach equally well to either lean or adipose tissue and, once attached, are equally difficult to remove. There is some evidence, based on electron micrographs, that bacterial cells may collect in crevices in the lean tissue. This might provide some physical protection from sanitizing agents, in that water trapped in the crevices may not allow direct contact between the sanitizer and bacterial cell. Alternately, water soluble components in the lean tissue may act to buffer the acid and chemically protect the bacteria. The practical implications of this are that much of the carcass surface is covered with adipose tissue, so the greater reductions on adipose tissue may be more like the reductions on actual carcasses.

Construction of the pre-evisceration washer in the abattoir at MARC is expected to be completed in the near future. The post-evisceration washer is operational, and the spray chiller is functional, although some additional refinements are required. With the completion of these systems, research under actual processing conditions will be performed to confirm the laboratory findings.

Predicting the Growth of Salmonellae on Beef

James S. Dickson, Gregory R. Siragusa, and James E. Wray, Jr. [1,2]

Introduction

Bacterial contamination of fresh meats can occur during normal slaughter and handling procedures, although this contamination can be minimized by adhering to good hygienic practices during slaughter. Since the bacteria are confined almost exclusively to the carcass surface as compared to the deep muscle tissue, procedures which can control the survival and growth of bacteria on tissue surfaces are of interest to both the meat industry and regulatory agencies. Chilling, either by forced air or water spray systems, is used universally to reduce the growth of bacteria on animal carcasses. However, because of the initial heat in an animal carcass, it takes several hours for the temperature to fall low enough to prevent bacterial growth. During the carcass cooling process, the contaminating bacteria can grow, resulting in a bacteria population many times greater than that of the initial contamination.

Bacterial growth progresses through several distinct phases. The first phase, called the lag phase, occurs as the bacteria adjust to a new environment. Although the bacteria are metabolically active during this phase, there is no net increase in numbers of bacteria. The second phase of growth is the logarithmic growth phase, where there is a rapid increase in the bacterial population. Eventually, the bacterial population exhausts most of the available nutrients and reaches a stable population, called the stationary phase. When graphed, the growth of bacteria resembles an "S" curve. The time required for a bacterial population to move from a static population (lag phase) to an actively growing state (logarithmic growth) is defined as the lag time. The time required for the bacterial population to double during the logarithmic growth phase is referred to as the generation time.

Bacteria generally have shorter lag and generation times as temperature increases, with the optimum temperature for bacteria of public health significance being very close to that of the body temperature of a cow (approximately 100-104°F). Since temperature has a significant effect on bacterial growth rate, the temperature history of food products has been used to estimate the potential bacterial population on a given product or, in practice, to predict relative rates of microbial growth for different cooling processes, with this process currently being referred to as temperature function integration. Although much of the previous research has focused on spoilage, this area also has applications for foodborne bacteria of public health significance.

The temperature function integration technique has been used for assessing beef carcass cooling processes. Researchers have used the temperature history of beef carcasses to predict the growth of *E. coli* during cooling, based on the growth of the bacterium in liquid cultures. Our intent was to construct a predictive model for the growth of salmonellae using intact beef tissue, with the specific purpose of evaluating beef carcass cooling procedures.

Procedure

Bacterium. A typical strain of *Salmonella typhimurium* was grown and maintained in tryptic soy broth.

Tissue. Postrigor beef tissue was obtained as boneless trim from the abattoir at the MARC. The tissue was separated into lean and fat tissues, sliced into 0.2 in thick slices, sterilized with gamma radiation, and stored frozen until use. Prior to use, the slices were cut into squares and tempered to room temperature. Tissue produced in this manner had previously been determined to be representative of prerigor tissue, in terms of numbers of bacteria which would attach and the sensitivity of the attached bacteria to organic acids.

Experimental design. Tissue samples were inoculated by immersing in a diluted bacterial solution for 5 min, drained briefly, and then attached to sterile clips or hooks and suspended in sterile containers. Sterile distilled water was added to the containers to minimize dehydration of the samples. Samples were incubated at 59°F, 68°F, 77°F, 86°F, 95°F, and 104°F and analyzed at 2 hr intervals. These temperatures were chosen to reflect the range of growth temperatures which would be encountered during beef carcass cooling. Since the generation time of salmonellae at 50°F is approximately 14 hr, 59°F was the lowest temperature evaluated.

Model development. Bacterial populations were converted to log10 colony-forming units, and each temperature and tissue combination was independently replicated three times. Data from each individual growth curve were fitted to the Gompertz equation (a mathematical equation which describes an "S" shaped curve) using nonlinear regression. Lag and generation times were defined and calculated according to currently accepted practices. Lag and generation times were modeled as exponential decay functions of temperature, using the formula:

$$Y = D + E^{*}e^{-F[(0.555^{*}(T))-32]}$$

where Y is the lag or generation time, T is the temperature in °F, and D, E, and F are derived parameters (Table 1).

Validation. Tissue samples and bacteria were prepared as previously described and tempered to 104°F in an environmental incubator. The tissue samples were inoculated by immersion for 5 min at 104°F, drained briefly, and then suspended over sterile distilled water. The samples were cooled in the incubators at rates of 10.8°F and 16.2°F per hr by a stepwise reduction in the temperature by 3.6°F or 5.4°F every 20 min, respectively. The incubator typically equilibrated to the lower temperature within 2 minutes. The surface temperature of uninoculated samples was monitored every 20 min using a surface temperature thermometer. After the sample temperatures reached approximately 50°F, the bacterial populations were enumerated. Lag times were estimated as the numerical average of the calculated lag times based on the maximum and minimum temperatures during the first two hr of cooling. At the end of the estimated lag time, the predicted generation time was calculated for each subsequent recorded temperature. Bacterial populations at each time interval were calculated by linear interpolation. In practice, the surface temperature was recorded every 20 minutes.

Results

The estimated lag and generation times for both lean and fat tissue, based on the models, are shown in Table 2. The lag times were generally longer on fat tissue than on lean, especially at temperatures at or below 77°F. The cells

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[1]Dickson is a research food technologist, Meats Research Unit, MARC; Siragusa is a microbiologist, Meats Research Unit, MARC; and Wray is computing services manager, MARC.

[2]The full report of this work has been submitted to Appl. Environ. Microbiol.

apparently require more time to adjust to the environment on the tissue surface. The reduced moisture in fat tissue may have reduced the availability of nutrients present on the surface of fat tissue. In addition, the lipid material in the fat tissue increases in viscosity and solidifies as the temperature decreases, which may further reduce nutrient transfer.

Once the bacterial cells adapted to the environment on the fat tissue surface, the growth was generally more rapid, again with shorter generation times noted at or below 77°F.

The predicted lag times for *S. typhimurium* on lean and fat tissue surfaces were substantially higher than those reported by one researcher, although they were similar to those reported by two others. The differences are a result of both the experimental design and subsequent data analysis. The first researcher inoculated mutton with either *Escherichia coli* SF or *S. typhimurium* grown to stationary phase, processed the meat in a commercial blender, and then vacuum sealed thin films of the inoculated meat in polyvinyl chloride pouches. Growth curves were conducted by immersing these pouches in a controlled temperature water bath. The lag and generation times were determined by graphing the data, and then plotted according to the "square root" model. Blending the meat tissue ruptured the muscle cells, releasing moisture and nutrients which would be readily available for bacterial metabolism. This abundance of readily available nutrients would be expected to reduce the length of time which the bacteria would require to acclimate to the environment, with a resulting decrease in lag time.

The predicted lag times reported by the other two researchers were generally intermediate values between those derived for lean and fat tissue. One of these grew a strain of *E. coli* isolated from sheep liver in a synthetic meat medium, which consisted of brain heart infusion broth supplemented with hemolyzed whole blood and lactic acid, while the other grew several strains of *Salmonella* in tryptone soya broth. The lag times were modeled as a simple quadratic equation or as a Gompertz equation. As previously noted, the lag times on fat tissue at the lower temperatures tended to be longer than those on lean tissue or in broth media.

The predicted generation times for lean and fat tissue were very similar to those previously reported. The greatest range of values occurred at 59°F, where the reported generation times ranged from 4.44 hr to 1.54 hr (fat tissue). A previous report had indicated that the average generation times of salmonellae on chilled beef at 50°F, 54.5°F and 59°F were 13.87, 6.79, and 3.25 hr, respectively. However, there was considerable variation in the generation times between replications, especially at 50°F, where the range of values was 25.5 hr to 8.1 hr. At temperatures above 59°F, the range of predicted generation times was less than 0.5 hr, with smaller ranges as the temperature approached 104°F. The implication is that, in spite of the broad range of media and different bacterial species used in the different experiments, the growth rate of similar bacteria is determined primarily by temperature. The predicted generation times on lean tissue at temperatures at and above 77°F were slightly higher than those of the previously published reports and those on fat tissue.

Although the model uses static temperature growth data to predict growth under dynamic temperature conditions, validation studies demonstrated that the growth model closely predicted the observed population increase of *S. typhimurium* during cooling of beef tissue (Table 3). The predicted populations were slightly lower than the observed values, although there was generally no significant difference between the predicted and observed populations. It is likely that the frequency of the recorded temperatures will affect the relative precision of the estimated populations, with a higher degree of precision associated with more frequent temperature recording. An electronic data acquisition system could record temperatures much more accurately, at intervals as short as 1 minute. These studies indicate that equations for lag and generation times are relatively accurate, within experimental error.

In summary, we have achieved the objective of developing a predictive model for the growth of salmonellae on beef tissue surfaces during cooling. The model has application in the evaluation of beef carcass cooling systems, as well as furthering the understanding of bacterial growth kinetics during changing environmental conditions. Since the model is based simply on the temperature of the carcass, the necessary data for analysis can be easily collected in any meat processing facility. The initial level of bacteria can be selected to represent a "typical" level of contamination, as well as levels representing "severe" and "reduced" contamination. The model can demonstrate the potential improvement in product quality by improving sanitation and processing during slaughter operations.

Table 1—Derived parameters of the equations for lag and generation times, taking the form of Y = D + E*e -F[(0.555*(T))-32], where Y is the lag or generation time in hr, T is the temperature in °F and D, E, and F are constants in the formulae

Tissue	Parameter	Lag	Generation
Lean	D	1.72	0.188
	E	59.02	7.65
	F	0.12	0.09
Fat	D	1.68	0.257
	E	338.27	5.104
	F	0.167	0.092

Table 2—Comparison of derived lag and generation times on lean and fat beef tissue

Growth temperature	Lag time (hr)		Generation time (hr)	
	Lean	Fat	Lean	Fat
59	11.48	29.31	2.14	1.54
68	7.07	13.67	1.45	1.07
77	4.66	6.88	0.99	0.77
86	3.33	3.94	0.70	0.58
95	2.61	2.66	0.52	0.46
104	2.21	2.10	0.40	0.39

Table 3—Comparison of observed and predicted populations of *S. typhimurium* on lean and fat tissue after cooling at rates of 10.8°F or 16.2°F per hr. Average initial population before cooling $\log_{10}$ 7.15 (lean) and 7.02 (fat) colony forming units

Tissue	Cooling rate	$\log_{10}$ population increase[a]	
		Observed	Predicted
Lean	10.8	1.52[b]1	1.42[1]
	16.2	1.03[2]	0.81[2]
Fat	10.8	1.90[3]	1.72[3]
	16.2	1.18[4]	0.91[c]5

[a] Increase in bacterial population determined as ($\log_{10}$ final population) - ($\log_{10}$ initial population).

[b] Means within rows with identical superscripts are not significantly different (P>0.10)

[c] Means for fat tissue, 16.2°F cooling rate are significantly different at the P<0.10 level, but are not significantly different at P<0.05.

Use of Calcium Alginate to Immobilize Antimicrobial Agents on Beef Tissue

Gregory R. Siragusa and James S. Dickson[1,2]

Introduction

Even under the best of slaughtering and processing conditions, beef carcasses will become naturally contaminated with some bacteria from the animal's hide, hair, hooves, and the abattoir environment. This contamination is mostly composed of bacteria which are harmless, but which can ultimately cause spoilage of the beef. The shelflife of raw beef is largely determined by the numbers and types of these bacteria. Since some bacterial contamination will always be present on beef, it is desirable to reduce these numbers to decrease the rate of spoilage, increase refrigerated shelflife, and further ensure the microbiological safety of raw beef before consumption.

Any methods to reduce this bacterial contamination would greatly benefit the beef processing industry. Methods are currently used in the red meat industry to decontaminate the carcass. These include spraying of water or antimicrobial agents on the carcasses. Sprays include the use of dilute chlorine in the spray chill water or the application of dilute foodgrade acid sprays on the carcass before chilling. These acids are usually either acetic acid or lactic acid, both of which are commonly consumed food ingredients.

Any methods which would enhance the antimicrobial effect of the acid or antimicrobial agent would be a significant improvement to beef production. We have developed the idea of applying food grade acids (acetic and lactic acids) into an edible gel coating which could be sprayed onto the carcass surface before entering the chilling chamber. Gel coatings have been shown to decrease the amount of moisture loss of the carcass. Incorporating the antimicrobial agent in an edible gel on the carcass would possibly help reduce moisture loss and simultaneously reduce the amount of spoilage bacteria. Any methods which decrease the amount of spoilage bacteria will also reduce the numbers of any pathogenic bacteria which may be present such as *Listeria*, *Salmonella*, and pathogenic *Escherichia coli*. The purpose of this research was to test the use of alginate edible gels to coat a layer of antimicrobial agent on the carcass surface to reduce the numbers of bacteria.

Procedure

Materials and Methods. Sterilized lean and fat beef tissue sections (3.6 in^2 total surface area) were inoculated with the foodborne pathogen *Listeria monocytogenes* (Lm). Tissue samples were dipped in a solution of 1% sodium alginate. Food grade acids (2% acetic and 1.7% lactic acids) were prepared in solutions of calcium chloride. Calcium chloride causes a gel to form when applied to alginate solutions. This firm gel will adhere to the meat surface. The inoculated meat samples were transferred from the alginate to the acid/calcium chloride solution. At this step, a gel was formed on the meat surface which included the acid antimicrobial agent. The inoculated/treated samples were stored at 40°F.

Microbiological and Data Analysis. Samples were taken at days 0, 1, 3, and 7. *Listeria* was enumerated by blending the sample in acid neutralizing solution, diluting this sample, and plating on two different bacteriological growth media to enumerate the bacteria. Bacterial counts were converted to $\log_{10}$ per total piece of tissue. The amount of reduction in the bacterial population was calculated as the difference in $\log_{10}$ bacteria per tissue section from day 0 to the sampling date.

Results

Table 1 shows the log reductions in bacterial counts from day 0 to day 7 at 40°F on lean beef tissue. Organic acids immobilized or incorporated into a gel decreased the numbers of *Listeria monocytogenes* attached to the beef tissue more than the acids applied without alginate. This effect was not observed in the first three days of storage (see Figure 1). *Listeria monocytogenes* grew on the tissue that was untreated, treated with calcium chloride, or treated with alginate and no acid. The largest reduction in numbers was caused by the application of acetic acid in a calcium alginate gel. Lactic acid at a lower concentration than acetic (1.7% vs 2.0%) caused a comparable but lower reduction than did acetic (Table 1). Counts determined by using Tryptic Soy agar represent the total of bacteria which are metabolically healthy and possibly injured by the effect of the acids. Counts determined on Oxford Listeria agar represent the portion of the bacterial population which is only metabolically healthy (Table 1). This difference is important to the food microbiologist when testing food because the use of only a selective agar (e.g., Oxford Listeria agar) to count a specific species of bacteria can result in underestimating the actual number. Calcium chloride or alginate by itself did not significantly affect the population of the test bacteria.

In the case of pure fat tissue, whether or not the acid was applied in an alginate gel made no difference on the reduction of *Listeria monocytogenes* counts based on Tryptic Soy agar. In the case of selective counts determined by Oxford Listeria agar, there was some increased reduction due to the alginate application method, however, this was not a statistically significant difference. Overall, the reduction in bacterial counts on fat tissue started at day 0 and continued through day 7 (Figure 2).

Discussion

Immobilizing organic acids in alginate gels and applying to inoculated beef tissue enhanced the antimicrobial effect of the acids on lean beef tissue. Overall, the bactericidal effect of acid sanitizers immobilized in alginate gels was much more pronounced on lean beef tissue. Applying organic acids in alginate to pure fat tissue did not enhance the antimicrobial action of the acids. This is a problem which might be overcome by using either different antimicrobial agents, such as other food grade organic acids, mixtures of these acids or higher concentrations of these agents. In addition, using other gelling agents may offer advantages. However, in the case of *Listeria*, the organism did readily multiply on fat tissue.

The exact mechanism by which alginate gel application of acids enhanced the killing effect of the acids is not known. It is hypothesized that the gel offers a means of maintaining the acid in a moist environment, which is necessary for the inhibitory action of the acid.

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[1]Siragusa is a microbiologist, Meats Research Unit, MARC, and Dickson is a research food technologist, Meats Research Unit, MARC.

[2]The full report of this work was published in J. Food Sci. 57:293-296, 1992.

Using alginate/acid coatings might offer an alternative to spray chilling. The carcass would be encapsulated in a highly moist gel coating, and heat would still be conducted off of the carcass in the carcass chilling chamber. Also, moisture loss would be reduced since the gel retains water. The use of alginate gels as coatings to reduce moisture loss in red meat carcasses has already been demonstrated.

This method might potentially be used to increase the shelflife of sub-primal beef cuts or used in other segments of the beef processing. Additional research is needed to determine the effectiveness of other antimicrobial compounds when immobilized in alginate or other gelling agents applied to beef carcasses. Research is underway to test the process on carcasses.

Table 1—Log reduction[a] in viable counts of L. monocytogenes on lean beef tissue after 7 days at 5°C

Treatment	TSAYE Agar Alginate	No alginate
Acetic[b]	1.5	0.25
Lactic[b]	1.26	0.02
Alginate control[c]	0.01	-0.44
Control tissue[d]	-0.61	

Treatment	Oxford Agar Alginate	No alginate
Acetic[b]	1.56	0.78
Lactic[b]	2.08	0.14
Alginate control[c]	-0.23	-0.44
Control tissue[d]	-1.16	

[a] Difference in counts within each treatment between day 0 and day 7.
[b] See Materials and Methods section for explanation of treatments.
[c] Alginate control, Alginate = no acid applied in alginate dip. Alginate control, no Alginate = $CaCl_2$ dip only, no acid or alginate applied.
[d] Control tissue = inoculated, untreated lean tissue.

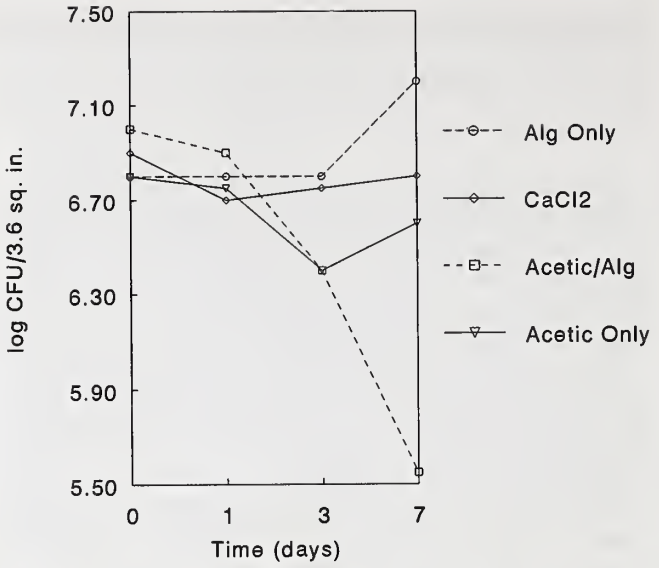

Figure 1—Reduction and growth of Listeria monocytogenes on lean beef tissue held at 40°F and treated with acetic acid applied by immobilizing in a calcium alginate gel. Bacterial counts were determined using Tryptic Soy agar medium.

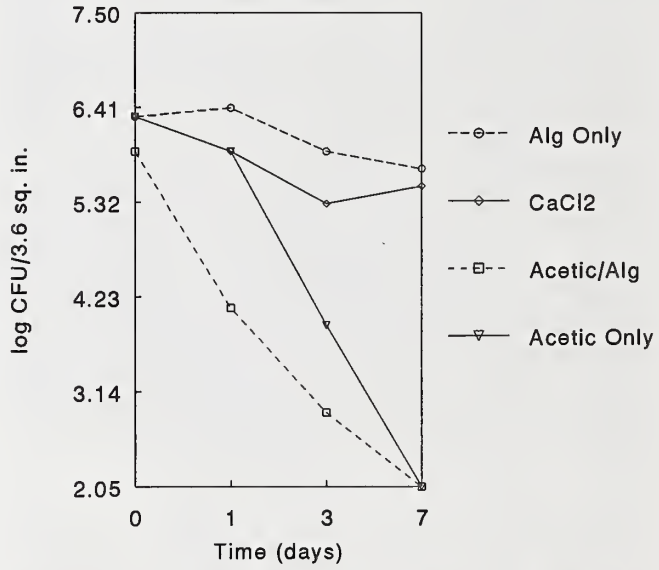

Figure 2—Reduction of Listeria monocytogenes on beef fat held at 40°F treated with acetic acid applied by immobilizing in a calcium alginate gel. Bacterial counts were determined using Tryptic Soy agar medium.

Comparisons of *Bos indicus* and *Bos taurus* Inheritance for Carcass Beef Characteristics and Meat Palatability

John D. Crouse, Larry V. Cundiff, Robert M. Koch, Mohammad Koohmaraie, and Steve C. Seideman[1]

Introduction

Crossbreeding is used widely to exploit heterosis and additive genetic variation among breeds to improve efficiency of beef production. The economic value of *Bos indicus* breeds of cattle, primarily Brahman, in crossbreeding programs in subtropical and tropical climates has been well established. In the temperate climatic conditions of MARC, productivity of *Bos indicus* x *Bos taurus* F_1 crossbred cows has been outstanding for reproduction and maternal performance relative to that of *Bos taurus* x *Bos taurus* F_1 cross cows when mated to produce terminal-cross calves by Red Poll or Simmental sires. (Brahman and Sahiwal are *Bos indicus* [humped] breeds; Pinzgauer, Angus, Hereford, Red Poll and Simmental and other European breeds are *Bos taurus* [nonhumped] breeds).

Palatability and leanness are important characteristics of beef that influence consumer demand. Tenderness is the dominant palatability attribute considered by consumers in determining meat acceptability. Previous research has shown that meat from *Bos indicus* breeds or crosses was less tender than meat from *Bos taurus* breeds or crosses of cattle. The objective of the research reported here was to determine the effects of 0, 25, 50 or 75% *Bos indicus* (Brahman or Sahiwal) inheritance on characteristics of carcasses and palatability of cooked meat.

Procedure

Carcass and meat traits of 422 steers differing in the ratio of Brahman (B), Sahiwal (S) or Pinzgauer (P) to Angus (A) or Hereford (H) inheritance were studied. Reciprocal backcross and F_2 matings (Table 1) provided calves with 0:100, 25:75; 50:50 and 75:25 ratios of Pinzgauer, Brahman and Sahiwal to Angus-Hereford inheritance. The steers were born in the spring of 1983, 1984, 1985 and 1986 in Cycle III of the Germplasm Evaluation (GPE) Program at MARC. After weaning in early October, steers were fed a growing ration until February. Subsequently they were fed, ad libitum, a mixed diet of corn silage, corn and soybean meal ranging in energy density from 2.74 Mcal of metabolizable energy per kg of dry matter early in the finishing period to 2.93 Mcal of metabolizable energy per kg of dry matter late in the finishing period.

The steers were slaughtered serially at two slaughter dates each year (avg interval was 40 days). All steers were about 13 to 15 mo of age at slaughter. After a 24 hr chill, carcasses were evaluated for USDA (1976) quality and yield grade criteria. Ribs were removed 24 hr postmortem, vacuum packaged, aged an additional 6 days at 34°F, frozen at -22°F and stored for up to 6 mo for subsequent shear force and sensory evaluation. An eight-member sensory panel was trained and tested. Panelists, in individual booths, evaluated three .5 in cubed samples for juiciness, ease of fragmentation, amount of connective tissue, overall tenderness, flavor intensity and off-flavor.

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Results

Carcass traits. Least squares means for final live wt and carcass traits for each of the 10 breed groups are given in Table 1. As expected from earlier phases of the experiment, Pinzgauer crosses were the heaviest (avg of all Pinzgauer crosses = 1122 lb), followed by Angus-Hereford crosses (1049 lb). The *Bos indicus* breed crosses were the lightest (avg of all Brahman crosses = 1014 lb; Sahiwal = 921). Results for wt are in contrast with results from the previous phase of the GPE program in which F_1 cross steers by Brahman sires were significantly heavier (87 lb) than F_1 Angus-Hereford cross steers. The sires used in the present study were the same as those used in the previous phase of the GPE program. Thus, a large portion of the increased wt advantage of the F_1 Brahman x Angus or Hereford may have been due to at least twice as much heterosis in *Bos indicus* x *Bos taurus* crosses as in *Bos taurus* x *Bos taurus* crosses, which has previously been reported by scientists in Texas (Cartwright and associates) and Florida (Koger and associates). The backcross and F_2 progeny in the present phase of the experiment are expected to have only half as much heterosis as that observed in the F_1 crosses. Thus, in backcross and F_2 matings, *Bos indicus* x *Bos taurus* crosses stand to lose at least twice as much from their heterosis effect as *Bos taurus* x *Bos taurus* crosses. These results suggest that heterosis effects, rather than additive gene effects, accounted for the superior growth observed earlier in F_1 crosses.

The Angus-Hereford crosses had the greatest fat thickness. Ribeye areas of Angus-Hereford crosses were similar to those of Brahman or Sahiwal crosses. The Pinzgauer breed crosses had the largest ribeye areas and least fat thickness. Percentage of *Bos indicus* had no consistent effect on fat thickness or ribeye area. *Bos indicus* crosses possessed a lower percentage of kidney, pelvic and heart fat than *Bos taurus* breed crosses.

The Angus-Hereford breed crosses had the greatest amount of marbling, followed by Pinzgauer crosses. *Bos indicus* crosses possessed the smallest amount of marbling, but the Brahman and Sahiwal crosses were similar to each other in marbling. Generally, marbling decreased as the percentage of *Bos indicus* inheritance increased.

Shear Force and Palatability Traits. Breed group avg of shear values and sensory panel scores are given in Table 2. Also, shear values greater than 6.95 (overall avg + 1 standard deviation) expressed as a percentage of the number of observations within each breed group are shown in Table 2. Significant variation among breed groups was observed in shear values, sensory panel ease-of-fragmentation scores, sensory panel perception of the amount of connective tissue and overall tenderness. Meat was observed to be similar for juiciness, intensity of beef flavor and off-flavor among breed groups.

Shear values increased and sensory panel estimates of tenderness decreased with increases in percentage of *Bos indicus* inheritance. Shear force required to slice through half inch cores of cooked rib steaks increased 1.6 lb for each 25% increase in Brahman inheritance and 2.9 lb for each 25% increase in Sahiwal inheritance. Sensory panel

estimates of tenderness decreased linearly as Brahman or Sahiwal germplasm was substituted for Angus or Hereford germplasm.

Separate analyses of variance and standard deviation for each breed group revealed that as the percentage of *Bos indicus* inheritance increased, within breed group variation in shear values increased. Standard deviations for shear were 2.2, 2.5, 2.6, and 4.7 lb for steers with 0, 25, 50 and 75% Brahman inheritance and 2.2, 3.7, 4.1, and 4.8 lb for steers with 0, 25, 50 and 75% Sahiwal inheritance, respectively. A similar trend was also noted for variation in sensory panel scores, especially in Brahman crosses. Percentage of Pinzgauer inheritance had no consistent effect on variation in shear values or overall tenderness.

The increased variation in shear and sensory panel tenderness scores resulted in increased percentages of shears greater than 15.3 lb and of sensory panel scores for tenderness of less than 4.6. Sensory panelists gave 41.2% of the meat from *Bos indicus* crossbreds tenderness scores of less than or equal to 4.62. As the percentage of Brahman or Sahiwal inheritance increased, the percentage of tenderness scores in the lower end of the scale increased. For 3/4 Sahiwal breed group, 85.7% of the tenderness scores fell within this range of the scale. Sensory panel scores for ease-of-fragmentation and amount of connective tissues also were less desirable as the percentage of *Bos indicus* inheritance increased. Percentage of Pinzgauer breeding had no effect on the tenderness observations, but shear values for Pinzgauer were greater than those for the Angus-Hereford cross group. Sensory panel scores for juiciness also decreased as the percentage of *Bos indicus* inheritance increased. Neither beef flavor nor off-flavor was affected by breed groups.

Implications. Obvious problems of tenderness exist in *Bos indicus* breeds of cattle. These problems seem to be independent of the environment in which animals were produced or composition of the meat. Problems associated with palatability must be solved before *Bos indicus* breeds of cattle can be used to optimize production efficiency in breeding programs without consideration of the negative impact of *Bos indicus* inheritance on meat palatability. Data reported here indicate that the tenderness problem probably is related to fragmentation of the muscle component of lean and, to a lesser extent, to theconnective tissue portion of lean. The biological basis of the variation in tenderness associated with *Bos indicus* breeds of cattle needs to be determined before solutions are likely to be found. In the mean time, breeding systems which optimize the *Bos indicus* influence at levels of about 50% in tropical environments (e.g., gulf coastal region) and about 25% percent in more subtropical to temperate environments are recommended to optimize performance levels for reproduction, maternal performance and other components of production efficiency and to more nearly match genetic potential to market requirements for beef with acceptable levels of tenderness.

Table 1—Carcass trait means for breed groups differing in ratios of Pinzgauer (P), Brahman (B) and Sahiwal (S) to Angus-Hereford (AH) inheritance

Breed group[a]	No.	Live wt lb	Carcass wt lb	Marbling score[b]	Fat thickness in.	Ribeye area sq. in.	Kidney, pelvic & heart fat
0:100 AH	107	1049	648	431	.57	10.80	3.0
25:75 P:AH	36	1120	687	421	.50	10.50	3.0
50:50 P:AH	44	1082	659	374	.37	11.80	2.8
75:25 P:AH	36	1164	701	366	.29	12.32	3.0
25:75 B:AH	28	1043	643	393	.47	10.82	2.9
50:50 B:AH	36	976	608	351	.43	10.62	2.8
75:25 B:AH	20	1022	633	306	.45	10.87	2.6
25:75 S:AH	35	1018	630	377	.42	11.07	2.7
50:50 S:AH	25	902	556	347	.42	10.23	2.8
75:25 S:AH	28	844	518	343	.37	10.00	2.6

[a] 0:100 AH denotes Angus (A) and Hereford (H) reciprocal backcross (HxAH, HxHA, AxAH, AxHA) and F_2 (F_1 x F_1 = AHxAH, HAxAH, AHxHA, HAxHA) progeny; Pinzgauer (P) breed groups include 25:75 backcross (AxPA, HxPH), 50:50 F_2 (PAxPA, PHxPH), and 75:25 backcross (PxPA, PxPH) progeny; Brahman (B) breed groups include 25:75 backcross (AxBA, HxBH), 50:50 F_2 (BAxBA, BHxBH), and 75:25 backcross (BxBA, BxBH) progeny; and Sahiwal (S) breed groups include 25:75 backcross (AxSA, HxSH), 50:50 F_2 (SAxSA, SHxSH), and 75:25 backcross (SxSA, SxSH) progeny.

[b] Marbling scored 300 through 399 = slight corresponds to USDA Select quality grade, and 400 through 499 = small corresponds to lowest one-third of the USDA Choice quality grade.

Table 2—Means for shear values and sensory panel scores of cooked ribeye steak samples from breed groups differing in ratios of Pinzgauer (P), Brahman (B), and Sahiwal (S) to Angus-Hereford (AH) inheritance

Breed group[a]	Shear		Sensory panel scores[c]						
						Tenderness			
	mean lb	>15.3[b] %	Juiciness	Ease of fragmentation	Connective tissue	mean	<4.6 %	Flavor intensity	Off flavor[d]
0:100 AH	9.7	1	5.2	5.4	5.2	5.4	6	5.0	2.8
25:75 P:AH	11.0	1	5.3	5.2	5.1	5.2	1	4.9	2.7
50:50 P:AH	11.3	4	5.2	5.3	5.1	5.3	2	5.0	2.8
75:25 P:AH	10.5	1	5.1	5.3	5.2	5.3	2	5.0	2.9
25:75 B:AH	11.4	7	5.2	5.2	5.0	5.2	7	5.0	2.9
50:50 B:AH	12.8	28	5.1	4.9	4.8	4.9	42	5.0	2.8
75:25 B:AH	14.7	70	4.9	4.6	4.4	4.5	55	4.9	2.9
25:75 S:AH	12.4	14	5.1	4.9	4.8	4.9	23	4.9	2.8
50:50 S:AH	14.6	48	5.0	4.6	4.6	4.6	60	4.9	2.9
75:25 S:AH	18.5	43	4.8	4.1	4.1	4.1	86	4.9	2.8

[a] See footnote a in Table 1.

[b] Shear values greater than 15.3 reflect the percentage of animals with tenderness values more than one standard deviation above the overall avg.

[c] Scored 1 = extremely dry, difficult, abundant, tough or bland through 8 = extremely juicy, easy, none, tender, or intense by a sensory panel.

[d] Scored 1 = intense through 4 = none by a sensory panel.

Effects of a β-Agonist on Muscle Protein Degradation, Enzyme Activity, and Meat Tenderness in Steers

Tommy L. Wheeler and Mohammad Koohmaraie[1,2,3]

Introduction

It is generally accepted that muscle proteins are under continual degradation during normal growth. It has been estimated that 15 to 22% of the animal's total energy expenditure is for this muscle protein turnover. Regulation of the rate of muscle protein degradation could cause dramatic changes in rate and efficiency of muscle growth. Despite their importance in muscle growth, the mechanisms and control of skeletal muscle protein degradation are unknown. It has been hypothesized that several enzyme systems are involved at different stages of degradation, and that the calpain enzyme system (which occurs naturally in muscle) may initiate protein degradation of the muscle fiber.

It has been demonstrated that β-agonists increase muscle growth. At least a part of this growth results from decreased protein degradation. β-agonists also decrease meat tenderness by increasing the activity of the calpain inhibitor, calpastatin. We know that the calpain system has a major role in postmortem tenderization of meat. We also hypothesize that the calpain system plays a major role in muscle protein degradation in the growing animal. The objective of this study was to determine the effects of a β-agonist on muscle protein degradation, muscle enzyme activity and meat tenderness of steers.

Procedure

Eight MARC III composite (1/4 Hereford, 1/4 Angus, 1/4 Pinzgauer and 1/4 Red Poll) steers weighing approximately 772 lb were randomly assigned to control or β-agonist fed treatment groups. Animals were allowed unrestricted access to a diet with or without 3 ppm of the β-agonist $L_{644,969}$ (from Merck Sharp and Dohme) in the diet. Two consecutive 24-hr urine collections were taken immediately before, and at 1, 3, 5, and 6 wk after β-agonist treatment began. Urinary concentration of N^{τ}-methylhistidine ($N^{\tau}MH$) and creatinine was measured. The skeletal muscle protein mass of the steers was estimated from urinary creatinine concentrations. $N^{\tau}MH$ is a modified amino acid found only in muscle (more than 90% in skeletal muscle) so it can be used to measure the rate and amount of skeletal muscle protein degradation. At the end of the 6 wk feeding period, the steers were slaughtered according to standard humane procedures.

Within 30 min postmortem, loin muscle samples were taken from the left sides for measuring the calpain enzymes and their inhibitor, calpastatin, and the lysosomal enzymes cathepsins B and B+L, and cystatin (cathepsin inhibitor). At 24 hr postmortem, the loin muscle from the right sides was removed and cut into five 1-in thick steaks and vacuum packaged. One steak each was assigned to 1, 3, 7, and 14 days postmortem aging. Loin muscle was obtained from each shear force steak for determination of the Myofibril Fragmentation Index (MFI), a measure of meat tenderness.

At the end of their respective aging times, the steaks were broiled to 158°F internal temperature. The steaks were chilled 24 hr, then .5-in diameter cores were removed parallel to the muscle fibers for the measurement of shear force (a mechanical measure of tenderness).

Results

The percentage of skeletal muscle protein degraded per day, called fractional degradation rate (FDR, percentage/day) was lower at 3 wk on trial and fractional accretion rate (FAR, percentage/day) of skeletal muscle protein was greater at 1, 3, 5, and 6 wk on trial compared to control steers (Table 1). However, fractional synthesis rate (FSR, percentage/day) was not different between treatment groups. FAR = FSR + FDR. Several researchers have shown a decrease in FDR as a result of β-agonist feeding, while others have inferred a reduction in FDR from a failure to find a change in FSR when accretion increased.

A convincing body of literature indicates that the calpain enzyme system has a major role in postmortem tenderization of meat. It also has been hypothesized that the calpains initiate muscle protein turnover by releasing myofilaments from the muscle fiber surface. Because β-agonists increase muscle growth, and reduce postmortem tenderization, it was of interest to determine the effects on the calpain enzyme system of feeding β-agonist. At 0 hr (30 min postmortem), μ-calpain and m-calpain activities were not different, but calpastatin activity was increased 60.0% in loin muscle from β-agonist fed steers (Table 2). After 7 days of storage at 35°F, μ-calpain and calpastatin activities had decreased relative to 0 hr, but again, only calpastatin was changed due to feeding the β-agonist (348% increase). These data on μ-calpain and calpastatin are consistent with previous findings.

Although not involved in postmortem tenderization, lysosomal cathepsins are hypothesized to be involved in protein turnover. However, there were no significant differences in cathepsin B, cathepsins B+L, or cystatin activities between control and β-agonist fed steers at 0 hr or 7 day postmortem (Table 2).There was a decrease in cathepsins B and B+L activities measured at 7 days compared to 0 hr postmortem. Previous data indicate very inconsistent effects of β-agonists on cathepsin activities, implying they are not involved in the rate limiting step of muscle protein degradation.

Consistent with the role of the calpain enzyme system in postmortem tenderization, tenderness was dramatically reduced in the loin muscle from β-agonist fed steers (Fig. 1). Shear force values decreased from 1 to 7 day postmortem in control steers. However, shear force of β-agonist fed steers did not vary from 1 to 14 day postmortem. In addition, MFI did not change in muscle from β-agonist fed steers from 1 to 14 day postmortem, although MFI increased from 1 to 7 day postmortem in control steers. The inhibition of calpain activity by the high calpastatin activity in the treated steers completely blocked normal postmortem tenderization.

The mechanisms responsible for the increased muscle growth are still very much in question. Increased synthesis, decreased degradation, or both, have been reported as a result of β-agonist feeding. Our results indicate fractional degradation rates (FDR) in treated steers had begun to decline after 1 wk, became significantly lower at 3 wk, but were again similar to controls by 6 wk of β-agonist feeding. These results are consistent with previous reports indicating

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[2]The authors would like to acknowledge the technical assistance of Peg Ekeren, Sue Hauver, Kay Theer, Pat Tammen, Bob Lee, Nels Johnson and Kathy Sorensen.

[3]The full report of this work will be published in the J. Anim. Sci., 1992.

the effects of β-agonists on protein degradation are rapid, and then decline. Other researchers have stated that β-agonists induce a short-term decrease in degradation and that as degradation returns to normal, protein synthesis increases. Estimated FSR was not different between control and treated steers. However, FSR was numerically higher at 5 and 6 wk. Thus, our data tend to support a diphasic effect of β-agonist on protein turnover. Considering the different β-agonists, species to which they are administered, varying dosages and time frames, and methodologies and time courses for measuring FSR and FDR, it is unlikely that all discrepancies will ever be completely reconciled. However, based on our data and a broad interpretation of previous data (to account for varying experimental conditions), it seems likely that β-agonists exert their effect on muscle growth through both decreased degradation and increased synthesis of skeletal muscle proteins. This is in contrast to increased growth of normal, rapidly growing muscle in which both synthesis and degradation are increased, although the former to a greater extent.

The role of various muscle enzymes in muscle protein turnover has not been determined. However, the calpain proteolytic system has been implicated in the initial, and possibly rate limiting, steps of muscle protein degradation. Furthermore, it has been clearly demonstrated that the calpain enzyme system is largely responsible for the observed variation in postmortem meat tenderness. Thus, it is believed that the calpain system could regulate muscle protein degradation during both muscle growth and postmortem storage of meat. In support of this hypothesis, it has been consistently demonstrated that β-agonist feeding increases muscle calpastatin activity, thereby reducing postmortem proteolysis by calpain and meat tenderness. Because postmortem proteolysis by calpain is reduced, it is conceivable that calpain proteolysis *in vivo* also might be reduced, thereby reducing the rate of muscle protein degradation (if calpain were involved in a rate limiting step). Our data are consistent with the previously reported increase in calpastatin activity and reduced meat tenderness resulting from β-agonist feeding. The combined observations of decreased calpain proteolytic capacity and reduced FDR in β-agonist fed steers compared to control animals supports this hypothesis. A greater understanding of the *in vivo* regulation of the calpain system is needed to further evaluate this hypothesis.

Table 1—Effects of β-agonist on skeletal muscle protein turnover of growing steers

	0 wk		1 wk		3 wk		5 wk		6 wk	
	Control	Treated	Control	Treated	Control	Treated	Control	Treated	Control	Treated
FDR[a], %/day	1.13	1.14	1.24	1.09	2.25	1.77*	1.81	1.77	1.41	1.56
FAR[b], %/day			.48	.63*	.32	.50*	.27	.45*	.12	.36*
FSR[c], %/day			1.72	1.72	2.57	2.27	2.08	2.22	1.53	1.92

[a] Fractional degradation rate of skeletal muscle protein.
[b] Fractional accretion rate of skeletal muscle protein.
[c] Fractional synthesis rate of skeletal muscle protein. FSR=FDR+FAR
* Treatment differences within week were significant at ($P < .05$).

Table 2—Effects of β-agonist feeding and time postmortem on muscle enzyme activities in loin muscle

	μ-Calpain[a]	m-Calpain[a]	Calpastatin[b]	Cathepsin B[c]	Cathepsins B+L[c]	Cystatin[d]
Control						
0 day	1.05	1.44	3.72	46.0	235.5	3.9
7 day	.02	1.27	.69	33.8	193.5	4.5
Treated[e]						
0 day	1.15	1.51	5.95*	36.5	215.5	4.3
7 day	.08	1.27	3.09*	26.8	181.5	4.6

[a] Low calcium requiring calpain.
[b] High calcium requiring calpain.
[c] Total activity.
[d] Measured as the ratio of B+L activity after to before cystatin(s) removal.
[e] 3 ppm of β-agonist for 6 wk.
* Treatment differences within postmortem time were significant ($P < .05$).

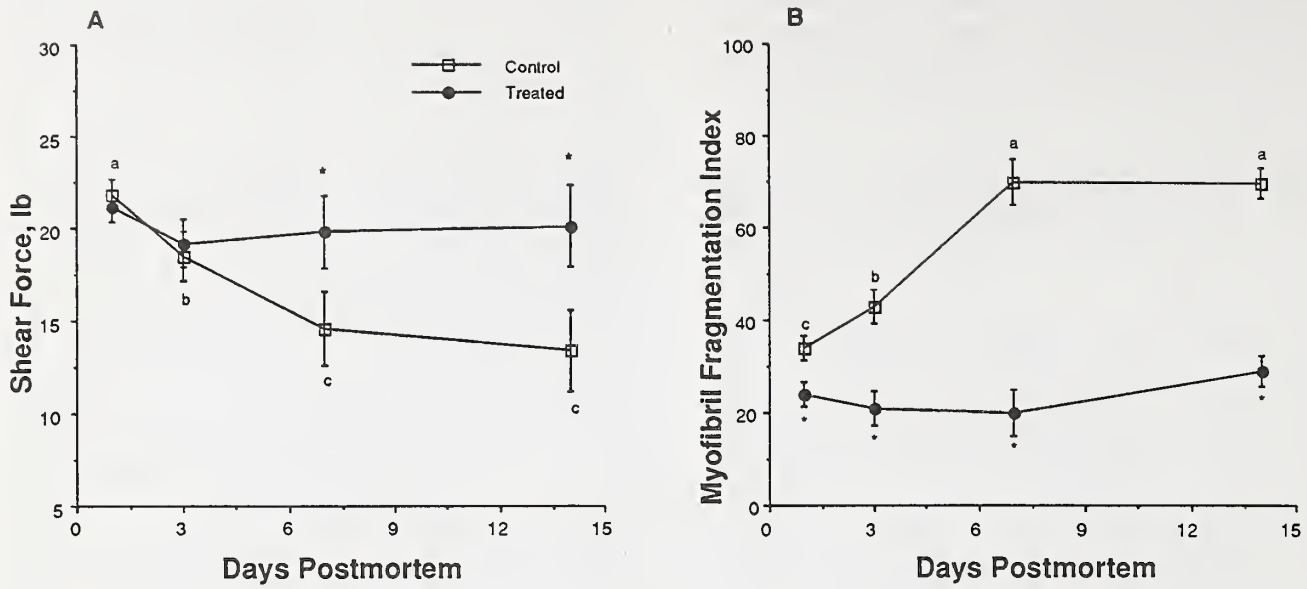

Figure 1 – The effects of feeding 3 ppm of β-agonist to growing steers for 6 wk on (A) shear force and (B) Myofibril Fragmentation Index during postmortem storage at 35°F.

* Treatment differences within postmortem time were significant ($P < .05$).

^{abc} Means for postmortem aging times within a treatment with a common superscript are not different ($P > .05$).

Meat Tenderness and the Calpain Enzyme System in Young Bulls and Steers

Tommy L. Wheeler, J. Brad Morgan, Mohammad Koohmaraie, Jeff W. Savell, and John D. Crouse[1,2]

Introduction

Comparisons of meat palatability between bulls and steers have indicated that meat from young bulls is more variable in tenderness. In addition, meat from bulls is usually numerically less tender, although the difference is frequently not statistically significant. This indicates that meat from bulls is only slightly, but consistently less tender than meat from steers.

Numerous studies have attempted to link bull meat toughness to higher amounts and decreased solubility of connective tissue. However, it has been reported that this change in bull meat does not occur until 12 to 16 mo of age. It has been shown conclusively, using different species and a variety of conditions, that the calpain enzyme system (which occurs naturally in muscle) is responsible for a majority of the tenderization that occurs during aging of meat. The possible contribution of the calpain system to differences in postmortem tenderization of muscle from bulls and steers has not been reported. This study was conducted to examine the effect of castration on palatability traits and 24-hr postmortem activities of μ-calpain, m-calpain and calpastatin in loin muscle of cattle.

Procedure

Six each, MARC III composite (¼ Red Poll, ¼ Pinzgauer, ¼ Hereford, and ¼ Angus) bulls and steers weighing approximately 397 lb were given unrestricted access to a growing/finishing diet. Animals were fed until 16 mo of age and slaughtered according to standard humane procedures. Carcasses were chilled for 24 hr at 34°F in the holding cooler.

At 24 hr postmortem, the loin muscle was cut into 1-in thick steaks and vacuum packaged. Steaks to be used for shear force (a mechanical measure of tenderness), Myofibril Fragmentation Index (a measure of tenderness), and trained sensory panel evaluation were assigned to 1, 7, or 14 days postmortem aging at 35°F. The steaks were stored at -50°F for 2 to 4 wk until they were thawed and cooked. Also at 24 hr postmortem, a sample of loin muscle was taken from each carcass for determinations of μ-calpain, m-calpain, and calpastatin activities.

Results

Conflicting reports exist concerning differences in tenderness of meat from carcasses of intact and castrated animals. Some researchers have reported that meat from bull carcasses is tougher and less palatable than meat from steer carcasses, while others have been unable to detect significant differences in tenderness of meat from young bulls and steers slaughtered at comparable ages. Our data indicate that regardless of postmortem aging time, bull loin muscle steaks had higher shear force values (i.e., less tender meat) than muscle from steer carcasses (Table 1). Steaks from steer carcasses had approximately a 30% decrease in shear force values between 1 and 14 days of aging. However, shear force values for bull loin steaks decreased only 20% after 14 days of aging. Loin tenderness is highly and positively correlated with Myofibril Fragmentation Index (MFI). Loin muscle from steers had higher MFI values than bull muscle at 1 day and 7 days postmortem, but not at 14 days (Table 1), indicating a greater amount of tenderization had occurred earlier postmortem in muscle from steers.

Sensory panel ratings indicated that as postmortem aging time increased, loin muscle samples became more tender with less detectable connective tissue (Table 1). It was anticipated that since shear force values indicated that meat from bull carcasses was tougher than meat from steer carcasses, differences in sensory tenderness ratings would be detected. However, gender had little effect on sensory panel myofibrillar or overall tenderness ratings (Table 1). In several previous reports involving assessment of palatability between bull and steer loin samples, meat from bull carcasses was tougher as indicated by increased shear force values; however, in most cases, the difference was small and more than likely would not result in consumer objection. Additionally, juiciness and flavor intensity were not affected by gender or time postmortem (Table 1).

A convincing body of literature indicates that the calpain enzyme system plays a major role in postmortem tenderization. The muscle proteins degraded by calpain enzymes closely mimic changes in muscle observed under normal postmortem aging. Our study showed there were no differences due to gender in 24-hr μ-calpain or m-calpain activities (Table 2). These data are consistent with previous findings indicating no differences in 24-hr calpain activities between *Bos taurus* and *Bos indicus* breeds of cattle. Previous research has indicated that under typical postmortem conditions, m-calpain is very stable, whereas there is a decline in the activity of μ-calpain and calpastatin (calpain inhibitor).

Calpastatin activity was 45% greater in loin muscle from bulls at 24-hr postmortem (Table 2). These data agree with the results of several recent experiments that indicate calpastatin is probably a primary regulator of m-calpain in postmortem muscle. Furthermore, the postmortem activity of calpastatin is highly related to the rate and extent of postmortem tenderization in meat from *Bos indicus* breeds of cattle, in meat from animals fed a β-agonist and in meat from different species. In the present study, calpastatin activity of meat from bulls at 24 hr postmortem (2.41 units of activity) was similar to the 0 hr calpastatin values for steers (2.24 units of activity; data not shown). The greater calpastatin activity in bull loin muscle could have decreased the amount of muscle protein proteolysis by μ-calpain, resulting in less tender meat.

Collectively, available evidence indicates that bulls are slightly, but consistently, less tender than steers, although the difference is frequently not statistically significant. Our data indicate that the rate of tenderization was greater in steers, implying that tenderness differences due to castration may depend on the time postmortem of measurement. Thus, this tenderness difference may not be of practical importance. We slaughtered our animals at 12 mo, thus, collagen solubility would not explain the tenderness differ-

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[2]The authors would like to acknowledge the technical assistance of Peg Ekeren (MARC) and Pansy Gilmore (TAMU).

ences we found. Furthermore, our data are consistent with the current hypothesis that higher calpastatin activity results in less tender meat due to decreased proteolysis by μ-calpain.

As previously reported, in tougher meat from *Bos indicus* breeds of cattle and animals fed β-agonists, the slightly tougher bull meat had higher calpastatin activity than meat from steers. These results support the theory that meat tenderness is inversely related to calpastatin activity. Calpastatin may regulate the activity of μ-calpain and in turn prevent muscle protein proteolysis and reduce ultimate meat tenderness. Research regarding meat tenderness and lean beef production systems should involve methods of manipulating the activity of the calpain inhibitor, calpastatin.

Table 1—Effects of postmortem aging time and gender on meat palatability traits

	1 day		7 days		14 days	
Trait	Bull	Steer	Bull	Steer	Bull	Steer
Shear force, lb	12.1[a]	11.2[b]	11.0[a]	9.3[b]	9.3[a]	7.5[b]
MFI[c]	22.0[b]	35.2[a]	53.5[b]	62.0[a]	72.6	73.2
Tenderness	4.0[b]	4.6[a]	4.7[b]	5.4[a]	6.2[b]	6.6[a]
Juiciness	5.2	5.1	5.1	4.9	5.1	5.2
Flavor intensity	4.6[b]	5.7[a]	5.4	5.7	5.8	5.9

[a b] Means in a row within aging time lacking a common superscript differ.

[c] Myofibril Fragmentation Index.

Table 2—Effect of gender on 24-hr calpain and calpastatin activities in loin muscle

Item	Bulls	Steers
μ-Calpain[a]	.29	.21
m-Calpain[b]	.80	.90
Calpastatin[c]	2.41[d]	1.33[e]

[a] Low calcium-requiring calpain.

[b] High calcium-requiring calpain.

[c] Inhibition of m-calpain.

[d e] Means within a row lacking a common superscript letter differ ($P < .05$).

Effect of Marbling Degree on Palatability and Caloric Content of Beef

Tommy L. Wheeler, Larry V. Cundiff, and Robert M. Koch[1,2]

Introduction

The relationship of marbling to beef palatability has been the subject of numerous investigations and several review papers. A vast majority of the data on this subject indicate that there is a positive relationship between marbling degree (or percentage chemical fat) and tenderness, juiciness, and flavor intensity, and an inverse relationship with Warner-Bratzler shear force (a mechanical measure of tenderness). However, this relationship is weak at best. Generally, although tenderness may increase linearly as marbling increases, the increments are very small, particularly from one marbling degree to the next. A comparison of the extremes in USDA quality grade (e.g., Standard and Prime) was usually needed to find statistical differences of any practical importance. Based on available data, it appears that between 5 and 10% of the variation in tenderness can be accounted for by USDA marbling degree. Most importantly, none of the studies detected palatability differences between Slight and Small marbling degrees that could justify price differentials frequently found in the market place. The objective of this study was to determine the effect of marbling score on palatability and caloric content of meat from diverse breeds of cattle.

Procedure

Animals. The data presented in this paper are from 1,337 steers and heifers from the Germplasm Evaluation (GPE) program at MARC. The breed groups represented include: Hereford, Angus, Longhorn, Salers, Galloway, Shorthorn, Piedmontese, Charolais, Gelbvieh, and Pinzgauer. These animals were born between 1986 and 1990 in March through May and weaned about October 1. After weaning, steers were fed a growing ration for 4 mo and then were allowed unrestricted access to a mixed diet of corn silage, corn and soybean meal. The cattle were slaughtered either at the MARC abattoir or at a commercial processing plant. After a 24 hr chill, the right sides of the carcasses from the commercial plant were transported to the meat laboratory at MARC at 48 hr postmortem. The loin muscle was removed and cut into 1-in thick steaks. The steaks were vacuum packaged and stored at 35°F until 7 days postmortem and then frozen at -86°F for up to 6 mo before thawing and cooking for Warner-Bratzler shear force and trained sensory evaluation.

Shear and Sensory Evaluation. Frozen steaks were tempered at 36°F for 24 hr then broiled to 158°F internal temperature (medium degree of doneness). The cooked steaks for shear force were chilled 24 hr at 36°F, then six .5-in diameter cores were removed parallel to the muscle fibers and sheared once each. Steaks for trained sensory evaluation were cut into .3 x .3 x 1-in samples and served warm to a trained sensory panel. Each panelist independently evaluated each sample for juiciness, tenderness, and flavor intensity on eight-point scales (1=extremely juicy or extremely tender or extremely intense; 8=extremely dry or extremely tough or extremely bland).

Proximate Analysis. Moisture content was determined by oven drying and chemical fat content by ether extraction on uncooked loin muscle. Protein content was calculated by difference, allowing 1% for ash content. Calories were calculated from the following equations:

1) Percentage protein x 4.46 x 28.4 = calories per oz protein
2) Percentage lipid x 9.01 x 28.4 = calories per oz fat

Results

Warner-Bratzler shear force was not different between marbling scores ranging from Slight through Moderate (Fig. 1A). Traces marbling was not different in shear force from Slight marbling, but had a higher shear force than Small, Modest or Moderate marbling scores. In addition, the percentage of meat with shear force of greater than or equal to 13.2 lb (comparable to an overall tenderness rating of 4.5 or "slightly tough") was similar between Small, Modest and Moderate marbling scores, but slightly higher for Traces and Slight. However, more than half of the meat with Traces or Slight marbling had shear force values comparable to "slightly tender" or better sensory tenderness rating. A similar response was found for tenderness rating (Fig. 1B). Meat with Traces marbling score received slightly lower tenderness ratings than Small, Modest, and Moderate marbling scores. The percentage of tenderness ratings less than 4.5 (slightly tough) was higher for Traces and Slight compared to Modest and Moderate marbling scores.

Juiciness rating tended to increase as marbling score increased, but Small marbling was not different in juiciness from any other marbling score (Fig. 1C). Meat with Traces or Slight marbling scores received lower juiciness ratings than meat with Modest or Moderate marbling scores. A slightly greater percentage of meat with Traces and Slight marbling scores received juiciness ratings of less than 4.5 compared to Modest marbling score. Beef flavor intensity was not affected by marbling score (Fig. 1D).

Regression of shear force and sensory traits on marbling indicated the inability of marbling score to predict meat palatability (data not shown). Equations for shear force, tenderness and juiciness ratings were significant, but only 1 to 3% of the variation in these traits was explained by marbling score. Clearly, marbling was of little value in explaining the variation in palatability of the meat in this study.

Percentage chemical fat, fat calories and total calories increased linearly as marbling score increased in uncooked loin muscle, except Traces was not different from Slight (Table 1). Percentage protein and calories from protein did not vary as marbling score increased. Percentage of total calories from fat increased and percentage of total calories from protein decreased as marbling score increased, except Traces was not different from Slight.

Due to the USDA quality grading standards and their implied segregation of meat based on palatability, the U.S. beef industry has placed a high value on marbling in the loin muscle. The emphasis on marbling in determining carcass value is based on the slight increases in juiciness, flavor and tenderness that are obtained as marbling is increased. There are, however, several problems with the current emphasis on marbling for segregating beef carcasses based on expected meat palatability. Firstly, an abundance of research stretching over the last 30 yr indicates that mar-

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[2]The authors would like to acknowledge the technical and statistical assistance of Kay Theer and Darrell Light.

bling fat has a low relationship to palatability and explains only about 5-10% of the variation in tenderness of the loin muscle. Secondly, other research indicates that the variation in marbling in the loin muscle has little or no effect on palatability of other muscles. Thus, a visual assessment of the amount of marbling in a cross section of the loin muscle at the 12th rib may not be appropriate as a major determinant of the value of the entire carcass. Our data support previous research indicating that marbling has little association with meat palatability. The emphasis on marbling in beef promotes excess fat production in cattle and does little to ensure desirable eating quality of the meat. Clearly, a more accurate method to predict meat palatability is needed.

Table 1—Composition and caloric content of 3.5 oz uncooked loin muscle with different marbling scores

	Chemical fat			Protein		Total	Calories	Calories
Marbling	N	%	Calories	%	Calories	calories	from fat, %	from protein, %
Traces	23	3.3[d]	30.3[d]	21.7	97.0	127.4[d]	23.3[d]	76.6[a]
Slight	456	3.5[d]	31.7[d]	21.9	97.6	129.3[d]	24.0[d]	76.0[a]
Small	661	4.7[c]	42.5[c]	21.6	96.2	138.7[c]	30.2[c]	69.8[b]
Modest	93	6.2[b]	55.9[b]	21.3	95.0	150.9[b]	36.8[b]	63.2[c]
Moderate	14	7.3[a]	65.5[a]	21.2	94.4	160.0[a]	40.5[a]	59.5[d]

[abcd] Means in a column lacking a common superscript are different ($P < .05$).

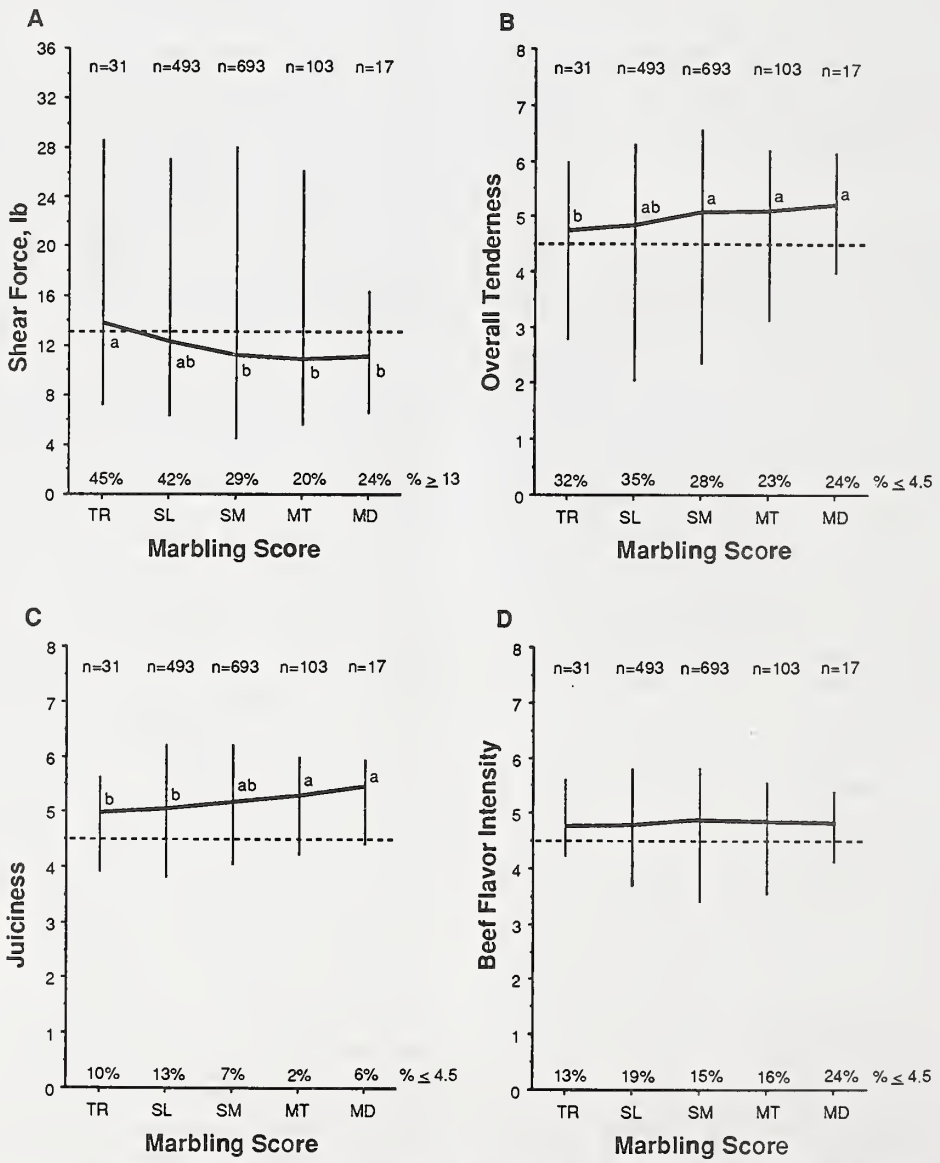

Figure 1 – Shear force and sensory traits as affected by marbling score. The darker, horizontal line passes through the mean values. The vertical lines represent the full range of values. The number of observations for each marbling score is given at the top. The percentage of samples that received unacceptable scores is given at the bottom. The broken line is the boundary between acceptable and unacceptable values. TR = Traces, SL = Slight, SM = Small, MT = Modest, MD = Moderate.

Effect of Castration on Skeletal Muscle Protein Turnover and Muscle Enzyme Activities in Cattle

Tommy L. Wheeler, J. Brad Morgan, Mohammad Koohmaraie, John D. Crouse, and Jeff W. Savell[1,2,3]

Introduction

It is well established that proteins are continually synthesized and degraded in skeletal muscle, but the proteolytic enzymes involved in muscle protein degradation remain unknown. It is hypothesized that the calpain proteolytic system, which is known to be important in postmortem protein degradation and thus in meat tenderization, could also be involved in or even possibly initiate muscle protein degradation in the living animal.

It is well documented that intact males grow more rapidly (15 to 17%), utilize feed more efficiently (10 to 13%) and produce higher yielding carcasses with less fat and more lean meat than castrates. However, the underlying mechanisms for these advantages have not been determined. The objective of this study was to determine the effect of gender (bull vs steer) on the relationship between muscle enzyme activity and muscle protein turnover in growing cattle.

Procedure

Six each MARC III composite (¼ Red Poll, ¼ Pinzgauer, ¼ Hereford and ¼ Angus) bulls and steers weighing approximately 397 lb were given unrestricted access to a growing/finishing diet. All animals were fed the experimental diet 5 wk before the initiation of the experiment to acclimate them to the diet. Two consecutive 24-hr urine collections were taken immediately before initiation of the experiment and at 42, 84, 126 and 168 days on feed. Urinary concentration of N^{τ}-methylhistidine ($N^{\tau}MH$) and creatinine were measured. The skeletal muscle protein mass of the steers was estimated from urinary creatinine concentrations. $N^{\tau}MH$ is a modified amino acid found only in muscle (more than 90% in skeletal muscle), so it can be used to measure the rate and amount of skeletal muscle protein degradation. At the end of the 168-day feeding period, the steers were slaughtered according to standard humane procedures.

Within 30 min postmortem, loin muscle samples were taken from the left sides for measuring the calpain enzymes and their inhibitor, calpastatin, and the lysosomal enzymes cathepsins B and B+L and cystatin (cathepsin inhibitor). Muscle for cathepsins was immediately frozen in liquid nitrogen and stored at -158°F until analyzed. Muscle for quantifying the calpain proteolytic system was immediately processed.

Results

Live animal performance data obtained in this study (Table 1) were similar to data reported previously for bull-steer comparisons. Bulls gained more rapidly than steers throughout the study. This advantage was statistically significant at both 84 and 168 days on test. Between the last two sampling periods (126 to 168 days), bulls grew more efficiently and to a heavier final body weight compared to steers (Table 1).

Fractional degradation rates (FDR) of skeletal muscle protein were lower in bulls than in steers at all sample times; however, those differences were statistically significant only at 168 days (Table 2). Fractional accretion rates (FAR) of skeletal muscle protein were not affected by gender, although bulls had numerically higher FAR at 84, 126, and 168 days. Steers were synthesizing more skeletal muscle protein per day than bulls at all times, although the differences were significant only at 168 days. The advantage in growth resulted because bulls were degrading approximately 30% less protein per day compared to steers.

No differences were detected in either μ- or m-calpain 0-hr activities between bulls and steers (Table 3). However, muscle calpastatin activity was greater for bulls than for steers. Another proteolytic system that may be involved in *in vivo* degradation of muscle proteins is the lysosomal enzyme system (cathepsins). Cathepsin B and cathepsins B+L activities were not affected by gender (Table 3). Results indicated that bull muscle contained more cystatin (cathepsin inhibitor) activity than muscle from steers.

Male cattle traditionally are castrated in the U.S., primarily to improve ease of management and palatability traits. However, young bulls have up to a 15% advantage in growth rate, feed efficiency and carcass leanness when compared with steers at the same age or time on feed. Many reports link the growth advantages associated with intact males to greater amounts of androgens such as testosterone.

The direct mechanism by which castration alters protein turnover remains unclear. In our study, improvements in muscle growth observed in bulls appeared to be related to decreased FDR. These results are in agreement with others who concluded that treating rats and lambs with testosterone increased muscle growth by suppressing muscle protein degradation. Additionally, female rats injected with a synthetic androgen, trenbolone, increased muscle gain primarily by reducing protein degradation. In addition, several reports have concluded that feeding β-agonists to growing animals increased muscle mass and improved whole body composition due to reductions in FDR. These results have been observed in lambs, rats, veal calves, chickens, rabbits and cattle.

MARC scientists have demonstrated that calpastatin is a powerful regulator of calpain-mediated proteolysis during postmortem aging. In fact, differences in the rate of postmortem proteolysis and tenderization of meat, regardless of species, are negatively correlated with the inhibitor of calpains, calpastatin. Several investigations substantiate the fact that animals with higher calpastatin activity produced meat which was tougher and exhibited less postmortem proteolysis compared to muscle displaying lower calpastatin activity. Unlike protein degradation occurring in postmortem muscle, very little is known about the mechanisms or factors which control or influence intracellular protein degradation in growing muscle. It has been proposed that the proteolytic capacity of the calpain system may regulate muscle protein degradation during both muscle growth and postmortem storage of meat. The lower FDR observed in bulls may be a

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[2]The authors would like to acknowledge the technical assistance of Peg Ekeren, Bob Lee, Nels Johnson and Kathy Sorensen.

[3]The full report of this work has been submitted to the J. Anim. Sci. for publication.

result of lower proteolytic capacity from calpain proteinases due to greater calpastatin activities. If calpastatin is related to protein turnover in living muscle, then an increase in calpastatin activity could possibly decrease calpain-mediated degradation and in turn reduce FDR. The significant negative correlation (r = -.72) between calpastatin and FDR (at 168 days) indicates that animals with higher calpastatin activities had lower FDR. Bulls exhibited higher calpastatin activities and decreased FDR compared to steers. We previously reported increased calpastatin activity was associated with decreased FDR in β-agonist fed steers.

Although no differences were observed in cathepsins B or B+L, greater cystatin activity was observed in bulls than in steers. Like calpastatin, a significant negative correlation (r = -.62) between cystatin and FDR was observed in our study. The relationship of cystatin to FDR may be in regulating cathepsin activity in later stages of muscle fiber disassembly.

Results suggest that the increased growth rate and efficiency of bulls compared to steers is partially due to increased protein muscle accretion resulting from reduced muscle protein degradation. Although no differences in m-calpain or m-calpain activities were observed between bulls and steers, the reduced proteolytic capacity of muscle due to increased calpastatin activity may serve as a regulator of muscle protein degradation. This information contributes to a better understanding of the complex mechanism and regulation of muscle protein metabolism that occurs in cattle.

Table 1—Effect of gender on animal performance traits

Trait and sample time[a]	Bulls	Steers
Live wt, lb		
0 day	470	463
42 day	600	571
84 day	738	681
126 day	855	796
168 day	1033	888
Avg daily gain, lb		
0 day	---	---
42 day	3.0	2.5
84 day	3.2	2.6
126 day	2.7	2.2
168 day	3.4	2.2
Feed/gain, lb/lb		
0 day	---	---
42 day	.24	.21
84 day	.28	.21
126 day	.20	.17
168 day	.15	.11

[a] Days from initiation of the study.

Table 2—Effect of gender on fractional degradation, accretion, and synthesis rates of skeletal muscle protein in growing bulls and steers

Trait and sample time[a]	Bulls	Steers
Fractional degradation rate, %/day		
0 day	1.64	1.80
42 day	1.45	1.78
84 day	1.41	1.80
126 day	1.83	2.26
168 day	1.30[c]	2.14[b]
Fractional accretion rate, %/day		
0 day	---	---
42 day	.37	.41
84 day	.35	.32
126 day	.28	.25
168 day	.29	.22
Fractional synthesis rate, %/day[d]		
0 day	---	---
42 day	1.82	2.19
84 day	1.76	2.12
126 day	2.11	2.51
168 day	1.59[c]	2.36[b]

[a] Days from initiation of the study.
[b c] Means in a row with different superscripts differ (P < .05).
[d] The summation of fractional degradation rate and fractional accretion rate.

Table 3—Effect of gender on 0 hr calpain enzyme system and cathepsin enzyme activities of loin muscle

Trait	Bulls	Steers
μ-Calpain[c]	1.32	1.22
m-Calpain[d]	.81	1.02
Calpastatin[e]	3.28[a]	2.24[b]
Cathepsin B[f]	28.47	32.17
Cathepsins B+L[f]	126.59	108.17
Cystatin[g]	3.84[a]	2.78[b]

[a b] Means in a row with different superscripts differ (P < .05).
[c] Low calcium-requiring calpain.
[d] High calcium-requiring calpain.
[e] Units of inhibition of m-calpain.
[f] Total activity.
[g] Measured as the ratio of B+L activity after to before cystatin removal.

Bos indicus Breeding Effects on Muscle Characteristics and Their Relationship With Meat Tenderness

Georgianna Whipple, Mohammad Koohmaraie, Michael E. Dikeman, John D. Crouse, and Melvin C. Hunt[1,2,3]

Introduction

Variation in meat tenderness that exists among animals may be due to genetics, diet, age, and other factors. Results from the Germplasm Evaluation (GPE) program show that rib eye steaks from many of the *Bos indicus* breeds of cattle are less tender than steaks from most of the European (*Bos taurus*) breeds of cattle, although *Bos indicus* crossbreeding programs are advantageous due to hybrid vigor and insect and heat resistance in subtropical regions. Because consumers consider tenderness to be the principal component of cooked beef quality, it is important to determine the biological factors that regulate meat tenderness. If this knowledge can be obtained, steps could be taken to decrease the variation in meat tenderness; thus, red meat producers could consistently provide consumers with a tender product.

Factors affecting meat tenderness have been studied by MARC scientists for many years. Some factors that may affect tenderness include muscle pH (acidity), rate of temperature decline after slaughter, muscle cell length (an indicator of the state of muscle contraction), the amount of total and soluble collagen (a form of connective tissue), muscle type (red vs white muscle types), and muscle enzyme activity. Of the many enzymes found in muscle, the calpain proteolytic (enzyme) system is thought to have a major role in the meat tenderization process. These calpain enzymes occur naturally in muscle tissue as well as a specific protein, known as calpastatin, that inhibits the activity of these enzymes. Two forms of calpain exist, μ-calpain which requires low calcium concentrations for activity, and m-calpain which requires high calcium concentrations for activity. Therefore, μ-calpain is the form that is active in muscle tissue after slaughter. During the aging or storage of meat, this enzyme system is active in degrading certain muscle proteins which must occur for meat to be tender. If the activity of this system is hindered in any way or potential for activity is lost, then tenderness is ultimately affected. Therefore, much emphasis has been placed on understanding this enzyme system.

This report summarizes results from experiments which were designed to determine which mechanisms associated with tenderness can best explain the differences or variation observed in tenderness between *Bos indicus* and *Bos taurus* breeds.

Procedures

Sahiwal crossbreds were used as the representative of the *Bos indicus* breed and Hereford X Angus crossbreds served as the contemporary *Bos taurus* breed. Seven heifers and four steers of 5/8 Sahiwal X Hereford, Angus or Hereford X Angus, three heifers and three steers of 3/8 Sahiwal, and five heifers and five steers of Hereford X Angus crosses were used in this study. Calves were weaned at 6 to 8 mo of age and fed an alfalfa haylage and corn silage finishing diet until 15 to 17 mo of age. Animals were then slaughtered over a 7 wk period. Carcasses were not electrically stimulated but were chilled at 30°F for 24 hr. Longissimus muscle (rib eye, loin) temperature and pH were determined at <1, 3, 6, 9, 12, and 24 hr after slaughter. Carcass data were collected at 24 hr. Samples were taken immediately after slaughter and 24 hr post-slaughter to determine activities of the calpain enzyme system. Besides the calpain system, the activities of another enzyme system (cathepsins) were determined. In addition, samples were taken to determine muscle type, muscle calcium and zinc concentrations, muscle cell length, and total and soluble collagen. The ease of muscle fiber fragmentation under controlled homogenization, which is an indicator of the amount of muscle proteins that have been degraded, also was determined. Also, we examined which muscle proteins were degraded. One inch thick rib eye steaks were obtained 14 days after slaughter to determine tenderness by measuring the amount of force needed to sever a 1/2 inch cooked meat core (Warner-Bratzler shear force). Cooked steak samples also were served to a trained sensory panel to determine tenderness, juiciness and flavor intensity.

Results

The *Bos indicus* and *Bos taurus* cattle used had similar USDA quality and yield grades, with averages of high Select and 3.2, respectively. No differences were found for lean color, lean firmness, lean texture, maturity scores, dressing percentage, and percentage of kidney, pelvic, and heart fat. Least-squares means for growth, carcass, and loin muscle chemical fat percentage are given in Table 1. Differences were observed for average daily gain, with the 5/8 Sahiwal crosses gaining most slowly. The Hereford x Angus crosses were significantly heavier at slaughter than the 5/8 Sahiwals. Small differences occurred for adjusted fat cover and rib eye area among the breed crosses. There were no statistically significant breed cross marbling differences, which contradicts some other reports, yet shows that quality grades are not totally indicative of tenderness. The chemical fat percentages support the marbling scores in that no breed cross differences were found.

Sensory panel evaluation scores and Warner-Bratzler shear force values (the greater the shear force the less tender the meat) for rib eye steaks aged 14 days are given in Table 2. The Hereford x Angus crosses were more tender as revealed by both lower Warner-Bratzler shear values and higher sensory panel tenderness scores. The sensory panelists found no differences in steak juiciness or flavor among the breed crosses. The muscle fiber fragmentation values (ease with which the sample fragments) were lower in the 5/8 Sahiwal meat samples (Table 2). The lower muscle fiber fragmentation values indicate that less muscle protein was degraded during the aging process in the 5/8 Sahiwal cross carcasses, which can explain why steaks obtained from their carcasses were less tender.

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[2]The full reports of this work were published in J. Anim. Sci. 68:2716-2728, 1990, and J. Anim. Sci. 68:4193-4199, 1990.

[3]The authors would like to express their gratitude for the technical support of Sue Hauver, Kathy Mihm, Pat Tammen, Kay Theer, and Jeff Waechter.

No breed cross differences occurred for temperature and pH decline after slaughter. Neither were there breed cross differences in muscle calcium and zinc concentrations, total and soluble collagen, muscle cell length, muscle type nor cathepsin enzyme activity (data not shown). Also, no differences in calpain activity were found among the breed crosses at either 0 hr or 24 hr postmortem (Table 3). Initial (0 hr) calpastatin activity (inhibitor of calpain activity) was similar among the breed crosses; however, at 24 hr, calpastatin activity was greater in muscle samples from 3/8 and 5/8 Sahiwal crosses (Table 3). The greater calpastatin activity may have inhibited calpain from degrading the muscle proteins that are necessary to make meat tender. This again is supported by the muscle fiber fragmentation values previously mentioned. Other research has shown that those muscle proteins degraded with postmortem aging of meat are also the muscle proteins degraded by calpain. In this study, when we evaluated which proteins were degraded, those proteins that are degraded in tender meat were also degraded in samples from Hereford x Angus crosses, but not in samples from the 5/8 Sahiwal crosses. Therefore, it appears that a lack of protein degradation is the reason why the Sahiwal steaks were less tender; and the greater 24 hr activity of calpastatin in the Sahiwal samples may be why this occurred.

Further statistical analyses were performed on 42 different variables obtained from this study to determine which variable(s) could accurately predict tenderness in this population of cattle. Of all variables, 24 hr calpastatin activity was able to explain 44% of the variation in tenderness. The variation in tenderness that was not explained by 24 hr calpastatin activity was explained partially by other variables. Muscle type accounted for 9 to 16%, muscle pH at 6 hr post-slaughter explained 9%, and cathepsin enzyme activity accounted for 10% of the tenderness variation. However, calpastatin 24 hr activity was the only variable that was significantly related to tenderness among and within breed subclasses.

These results suggest that differences in the amount of muscle protein degraded during the aging of meat is the major reason why steaks from Sahiwal crosses are less tender than steaks from Hereford x Angus crosses, and why the calpain-calpastatin system appears to be responsible for these differences. Therefore, knowledge of the calpain system mechanism and how it is regulated in postmortem muscle tissue is a must. Then, methodology could be developed to optimize the amount of muscle protein degraded through the action of the calpain system, thus ensuring tender meat. These methods may be through animal selection, dietary management, and/or post-slaughter handling practices.

Table 1—Least-squares means for growth, carcass and chemical traits by breed cross

	Breed cross		
Trait	H x A	3/8 Sah	5/8 Sah
ADG, lb	2.11	1.94	1.78
Slaughter wt, lb	1104.6	1030.9	986.2
Hot carcass wt, lb	678.9	644.8	622.8
Adj. fat thickness, in	.60	.55	.47
Rib eye area, in^2	11.63	11.01	10.94
Marbling	$Small^{51}$	$Slight^{76}$	$Small^{58}$
Rib eye chemical fat, %	5.4	4.0	4.7

Table 2—Least-squares means for Warner-Bratzler shear force, sensory-panel scores and muscle fiber fragmentation values

	Breed cross		
Trait	H x A	3/8 Sah	5/8 Sah
Warner-Bratzler shear,lb[a]	10.3	14.1	16.9
Sensory-panel score			
Tenderness[b]	5.9	5.0	4.4
Juiciness[c]	5.4	5.3	4.8
Flavor[d]	4.9	4.7	4.7
Muscle fiber fragmentation values[a]	82	75	60

[a] A higher shear force value indicates less tender meat.
[b] A score of 6=moderately tender,... 4=slightly tough.
[c] A score of 6=moderately juicy,... 4=slightly dry.
[d] A score of 6=moderately intense,... 4=slightly bland.
[a] A higher muscle fiber fragmentation value indicates that more protein has been degraded; thus, muscle fibers are easier to fragment.

Table 3—Least-squares means for calpain and calpastatin activity 0 and 24 hr post-slaughter

	Breed cross		
Trait	H x A	3/8 Sah	5/8 Sah
0 hr			
μ-Calpain	114	109	104
m-Calpain	106	92	97
Calpastatin	398	351	357
24 hr			
μ-Calpain	35	45	35
m-Calpain	107	116	118
Calpastatin	135	196	210

Acceleration of Postmortem Tenderization in Brahman-Cross Beef Carcasses by Calcium Chloride

Mohammad Koohmaraie, Georgianna Whipple, John D. Crouse[1,2]

Introduction

The recently completed National Tenderness Survey and Beef Quality Audit have clearly demonstrated that variation in beef tenderness at the consumer level is one of the major problems that face the meat industry. Because of this, and since consumers consider tenderness to be the principal component of meat quality, scientists in the Meats Research Unit of MARC have placed a special emphasis on understanding factors that determine beef tenderness.

To enhance tenderness, meat is normally aged (as wholesale cuts or carcasses). During this aging period a number of changes occur in the meat which result in loss of its strength. This is translated into less resistance during the chewing of meat after cooking; therefore, tenderness is improved. Over the past decade, we have determined the cause of the tenderization process during cooler aging. Meat is composed of long fibers that are held together by a rope-like structured protein called desmin. During cooler aging, this protein is broken down by naturally occurring enzymes called calpains. The amount of calpain activity will determine the extent of improvement in tenderness with aging. Calpain is a unique enzyme system which can degrade proteins only when sufficient calcium is present. With this knowledge, MARC scientists have developed a procedure that produces tender meat at its maximum level only one day after slaughter. The procedure involves infusion of carcasses, or injection of meat cuts, immediately after slaughter with a calcium chloride solution (3.3%). The addition of calcium chloride causes maximum activation of the calpain system; therefore, maximum tenderization occurs in a short time.

Historically, crossbreeding has been widely used as a means of improving efficiency of beef production. The economical value of *Bos indicus* breeds of cattle in crossbreeding programs in semitropical and tropical climates has been well established. One of the major problems associated with inclusion of *Bos indicus* cattle in cross-breeding programs is that meat from these cattle has objectionable tenderness ratings. Previous research by MARC scientists indicates that the reason for meat tenderness problems associated with *Bos indicus* carcasses is lack of tenderization during cooler aging. Because we had demonstrated the effectiveness of calcium chloride in improving meat tenderness, the objective of this experiment was to determine whether calcium chloride injection could improve tenderness of meat from *Bos indicus* carcasses.

Procedures

Twelve 5/8 Brahman x Hereford or Angus (four steers and eight heifers, about 18 mo of age) were slaughtered in MARC's abattoir according to standard procedures. Within 45 min of slaughter (time required for slaughtering and evisceration), a section of the loin muscle (back muscle) 15 inches in length (from first to sixth lumbar vertebra) from one side of the carcasses was needle-injected with a calcium chloride solution (3.3%). The volume of the injection was 18 oz. The loin muscle from the other side of the carcass served as the control (not injected). After completion of the injection process, the sides were transferred to a cooler (30°F). After 24 hr at 30°F, the loins from the injected and noninjected sides were removed for tenderness determinations. Loin steaks from injected and noninjected sides were cooked after one and fourteen days after slaughter.

Results

Carcass characteristics of the animals used in this study are reported in Table 1, and results of calcium chloride injection are reported in Table 2. Consistent with our previous observations of calcium chloride infusion of lamb carcasses, calcium chloride injection of beef loin muscle resulted in a significant elevation in muscle calcium concentration, and an acceleration of postmortem tenderization as determined by shear force (the amount of force required to penetrate a .5-in core of cooked steak). The results presented in Table 2 also demonstrate that calcium chloride improves meat tenderness by activating these naturally occurring calpain enzymes. One of the unique characteristics of calcium chloride-induced tenderization is that meat is never overtenderized. The reason is that during the process of breaking down muscle proteins (particularly desmin) to improve meat tenderness, these enzymes will also degrade themselves. These results, therefore, demonstrate that calcium chloride injection is an effective method of providing tender meat from carcasses producing meat with unacceptable tenderness.

This technology (calcium chloride injection/infusion) could have a significant effect on the meat industry. However, the usefulness of this process will depend on successful modification to allow practical application by the meat industry. We are now addressing some of the important questions that must be answered prior to making any recommendation to the industry. Some of these issues include: concentration of calcium, volume to be injected, and effects on meat color and flavor. Following are some important characteristics of calcium chloride infusion/injection that deserve special attention: (1) the maximum tenderness value is obtained within one day postmortem; (2) the process consistently produces uniformly tender meat; (3) because of the unique built-in control, meat is never overtenderized as with other enzymes [e.g., papain]; (4) because calcium accelerates the rate of rigor onset [shortly after calcium chloride infusion or injection of prerigor meat, the rigor process is completed], the process can easily be applied to hot-boning, which has traditionally been considered as a means of decreasing energy and labor costs associated with chilling and fabricating carcasses, and (4) from a human nutrition standpoint, this process can be used as a method of increasing calcium intake. The importance of calcium in the human diet is well recognized. In fact, some suggest that because meat is widely consumed, calcium fortification of processed meat is one of the best possible methods of increasing calcium intake in the population.

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[1]Koohmaraie is the research leader, and Whipple is a research associate, Meats Research Unit, MARC. Crouse is assistant area director, ARS, USDA, Northern Plains Area, formerly the research leader, Meats Research Unit, MARC.

[2]The full report of this work was published in J. Anim. Sci. 68:1278-1283, 1990.

Table 1—Bovine carcass characteristics

Trait	Mean	SD	Minimum	Maximum
Hot carcass weight, lb	734	46	552	895
Lean color[a]	4.17	.58	3	5
Lean firmness[b]	6.42	.9	5	8
Lean texture[c]	5.92	.9	4	7
Lean maturity[d]	149.2	6.7	140	160
Skeletal maturity[d]	157.5	3.3	140	170
Overall maturity[d]	153.3	2.2	140	165
Marbling[e]	387	60	310	530
Adjusted fat thickness (in)	.57	.16	.3	.9
Ribeye area (in^2)	11	1	9.7	12.6
Estimated kidney, heart and pelvic fat (%)	3.29	.78	2	4.5
Yield grade	3.77	.78	2.2	4.9

[a b c] Scored: 1 = very dark, soft, or coarse, through 8 = very light cherry red, very firm, or very fine.

[d] Scored: 100 through 199 = A.

[e] Scored: 300 through 399 = slight, 400 through 499 = small, and 500 through 599 = modest.

Table 2—Effect of calcium chloride injection of beef loins on calcium content, cooking parameters and enzyme activities

	Control		Calcium-injected		Probability levels		
Trait	d 1	d 14	d 1	d 14	Treatment	Aging	Interaction
Water-extractable calcium (ppm)	11	1,346	.01				
Shear force (lb)	19.9	13.7	13.2	11	.01	.01	.01
Cooking loss (%)	19.36	20.47	25.09	22.5	.01	.28	.01
Cooking rate (min/oz)	3	3	3	3	.56	.8	.58
μ-Calpain[a]	62.1		1.9		.01		
m-Calpain[b]	131.4		34.9		.01		
Calpastatin[c]	166.1		17.3		.01		

[a] Low-calcium-requiring calcium-dependent protease. Total activity/.22 lb muscle (caseinolytic assay).

[b] High-calcium-requiring calcium-dependent protease. Total activity/.22 lb muscle (caseinolytic assay).

[c] Total activity/.22 lb muscle (inhibition of casein hydrolysis by m-calpain).

A Calcium Chloride Injection Process to Produce Guaranteed Tender and Calcium Fortified Meat

Tommy L. Wheeler, Mohammad Koohmaraie, and John D. Crouse[1,2,3]

Introduction

The recently completed National Beef Tenderness Survey revealed that current beef production practices result in considerable variation in meat tenderness and an unacceptable percentage of tough meat, particularly round and chuck cuts. It has been known for several years that meat from *Bos indicus* cattle was tougher than meat from *Bos taurus* cattle and that *Bos indicus*-influenced cattle make up approximately 25% of the beef cattle in the U.S. In addition, as the beef industry moves towards leaner beef, many production systems that decrease fatness also result in decreased tenderness (i.e., bulls vs steers, forage feeding, growth promotants). Furthermore, the 1992 Beef Quality Audit reported that retailers, restaurateurs and meat purveyors all listed tenderness as one of their top ten problems. Clearly, some means of improving and ensuring meat tenderness is needed.

A potential solution to this problem already exists. It has been demonstrated by MARC scientists that meat tenderness can be improved dramatically at 1 day postmortem by infusing whole carcasses or injecting specific cuts with a calcium chloride solution within 1 hr postmortem. The addition of calcium activates a naturally occurring enzyme (calpain) in the muscle which accelerates postmortem tenderization so that uniformly tender meat is consistently obtained at 1 day postmortem. This process has been used successfully in normal lambs, lambs fed a β-agonist, *Bos indicus* cattle and 12-yr old cows.

However, industry adoption of this technology will probably require hot-boning for prerigor injection or may be more likely if it could be applied after 24 hr chilling, rather than immediately postmortem, in order to avoid conflict with inspection and grading procedures. Thus, several experiments were conducted to evaluate the use of calcium chloride injection in hot-boned prerigor muscles (at 30 min postmortem) and to evaluate the application of the injection process in postrigor meat (at 24 hr postmortem).

Procedure

Experiment 1. Fifteen *Bos indicus* bulls (3/8, 1/2 or 5/8 Brahman or Sahiwal x Angus or Hereford) were fed a growing diet and slaughtered at 18 mo of age weighing 1215 lb. The bottom round muscles were hot-boned from seven carcasses at 30 min postmortem. Eight carcasses were left intact, chilled and the bottom round muscles removed at 24 hr. The muscles from the right sides served as controls. The bottom round muscles from the left sides were injected with a 3.3% calcium chloride solution at 10% by weight.

Experiment 2. Nine *Bos indicus* steers (3/8, 1/2 or 5/8 Brahman or Sahiwal x Angus or Hereford) were fed the same diet as in Exp. 1 and slaughtered at 19 mo of age weighing 1206 lb. The top round muscles were hot-boned from both sides of each carcass at 30 min postmortem. The muscles from the right sides served as controls and the muscles from the left sides were injected with calcium chloride as described above.

Six steaks were cut 1 in thick from all treatments, vacuum packaged and two steaks each were aged either 1, 8 or 14 days postmortem at 35°F. At the end of the respective aging periods steaks were broiled to 158°F internal temperature, chilled 24 hr at 35°F then .5-in cores were removed parallel to the muscle fibers to determine shear force (a mechanical measure of tenderness: higher shear force = tougher meat).

Experiment 3. Seven *Bos indicus* crossbred bulls (3/8, 1/2 or 5/8 Brahman or Sahiwal x Angus or Hereford) were fed as described above and slaughtered at 16 mo of age weighing 1276 lb. The longissimus muscle was removed from one side of each carcass at 30 min postmortem and divided into the following treatments: 1) aged 7 days, 2) injected immediately, aged 7 days, 3) injected day 1, aged 7 days, 4) frozen day 1, thawed, aged 7 days, and 5) frozen day 1, thawed, injected, aged 7 days. Injected treatments were injected at 10% by weight with a 3.3% calcium chloride solution with a hand stitch pump. Frozen treatments were frozen at -86°F for 7 days. All treatments received a total of 7 days of postmortem aging at 35°F, then steaks were cut, broiled and sheared as described above.

Results

In Exp. 1, calcium chloride injection of bottom round muscles from carcasses of Brahman crossbred bulls significantly reduced shear force requirements (improved tenderness) at all postmortem aging times (Table 1). Postmortem aging necessary to ensure tender meat was reduced to 1 day. Even after 14 days of postmortem aging calcium injected meat was more tender than the control meat. Hot-boning had no effect on shear force of bottom round muscles.

Because hot-boning did not affect shear force in Exp. 1, all top round muscles were hot-boned in Exp. 2. As with the bottom round muscles, calcium injection resulted in significant reduction in shear force requirements of top round muscles at all postmortem aging times (Table 1). Even though the control top round muscles were initially tougher than the bottom round muscles, the calcium injection was equally successful at increasing tenderness by 1 day postmortem.

Research has previously shown that calcium injection of prerigor meat dramatically tenderizes longissimus muscle. All research to date in this area indicates that 1 day postmortem shear force requirements are consistently reduced to 6 or 7 lb by increasing the calcium concentration of the muscle immediately postmortem. Industry adoption of this process would probably require that it be coupled to hot-boning so that the injection could be applied to meat soon after slaughter. Hot-boning of beef has traditionally been considered as a means of decreasing energy and labor costs associated with chilling and fabricating beef carcasses. However, its use has not been accepted by the beef industry. It has been reported that tenderness of hot-boned meat was sometimes decreased. This decrease varied greatly, though, depending on the conditions of the hot-boning process. Because our data indicate that hot-boning

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[2]The authors would like to acknowledge the technical assistance of Kay Theer and Pat Tammen.

[3]The full report of this work will be published in the J. Anim. Sci., 1992.

had no detrimental effect on tenderness, and that if it did it would be more than offset by calcium injection, hot-boning could be used to facilitate injection of calcium chloride, thereby increasing meat tenderness and enhancing the potential use of hot-boning in the beef industry.

However, the adoption of this technology by the meat industry may be hampered by injecting the exogenous calcium into prerigor muscle due to interference with USDA inspection. It would logistically be much easier to inject a calcium chloride solution into meat after 24 hr postmortem, such as at the end of the fabrication line, before vacuum packaging and boxing. However, it is possible that changes in cell membrane permeability during rigor development could restrict the entry of calcium into the muscle cell and thus prevent the activation of the enzyme (calpain). If this were the case, some additional means of ensuring that sufficient calcium enters the cell to activate the enzyme (calpain) would be needed. This might be accomplished by freezing the meat first, then thawing and injecting, since freezing creates ice crystals large enough to cause structural damage to the muscle cell membrane and could facilitate calcium uptake by the cell and enzyme (calpain) activation. In addition, frozen storage has been shown to decrease the activity of the calpain inhibitor (calpastatin). Thus, injecting previously frozen and thawed meat with calcium chloride may enhance tenderization by increasing calpain activity through increasing access to calcium and(or) reducing calpain inhibitor (calpastatin) activity.

Experiment 3 was conducted to determine whether injecting postrigor meat with calcium chloride would result in similar tenderization as with prerigor injection. Meat from the control treatment with 7 days postmortem aging was less tender than other treatments (Table 2). Freezing alone induced an intermediate reduction in shear force. Injecting calcium immediately after slaughter or at 1 day postmortem, with or without prior freezing, resulted in similar and dramatic reductions in shear force requirements at 7 days postmortem. Thus, freezing and thawing before injecting postrigor meat with calcium chloride was not necessary to obtain similar tenderization as with injection of prerigor meat. This experiment confirmed that postrigor calcium chloride injection tenderizes meat as well as prerigor injection if aged until 7 days postmortem. The freeze then thaw and inject treatment was not necessary to obtain tender meat. The National Beef Tenderness Survey indicated that the earliest meat was displayed in the retail case was 7 days postmortem, so ensuring that meat will be tender by 7 days postmortem is sufficient.

These data indicate that either pre- or postrigor injection of calcium chloride could be used to improve meat tenderness, depending on the individual operating conditions. Furthermore, prerigor injection could be used in conjunction with hot-boning to reduce processing time and costs. In addition, this process would never overtenderize (such as sometimes occurred with the enzyme papain) because calpain also degrades itself and loses activity. In addition, calcium chloride has been approved by FDA as GRAS (Generally Recognized as Safe) at maximum levels of 3% of an .8 M solution. These data indicate the potential for ensuring that virtually all meat is very tender, regardless of the source. Thus, calcium injected meat could be marketed as calcium fortified and guaranteed tender.

Table 1—Effect of calcium chloride injection and hot-boning on shear force (lb) of top and bottom round muscles

	Bottom round				Top round	
	Intact		Hot-boned		Hot-boned	
Aging time, days	Control	Calcium	Control	Calcium	Control	Calcium
1	12.4	8.0	14.1	8.2	19.8	8.1
8	10.2	8.4	12.1	7.4	18.8	7.0
14	10.1	7.2	10.8	8.1	16.1	7.1

* Calcium-injected meat was more tender ($P < .05$) than the control in all comparisons.

Table 2—Effect of time of calcium chloride injection and freezing on shear force of the loin muscle

Treatment[a]	Total aging time, days	Shear force, lb
Control	7	16.0[b]
0 hr inject	7	6.2[d]
1 day inject	7	8.5[d]
Freeze	7	11.3[c]
Freeze/inject	7	5.6[d]

[a] See procedures for detail regarding treatments.

[bcd] Means in a column lacking a common superscript letter differ ($P < .05$).

Improving Beef Tenderness With Calcium Marination

Georgianna Whipple and Mohammad Koohmaraie[1,2]

Introduction

Red meat consumers consider tenderness to be the principal component of meat quality. However, variation in tenderness often occurs among cattle breeds as well as different muscles. MARC scientists have developed a process that accelerates the meat tenderization process that occurs with post-slaughter aging. This developed process involves the injection of meat cuts with calcium chloride (see previous article on calcium chloride injection). However, a different calcium application method, such as marinades, may prove to be of greater use if successful in improving tenderness. Currently, marination is widely used by consumers to improve meat tenderness and flavor. The use of calcium in marinades may improve tenderness by activating the natural calpain enzymes found in muscle tissue, because calpain requires calcium to be active. Sufficient calcium concentrations are reached post-slaughter to activate the low-calcium-requiring form, μ-calpain, but not the high-calcium-requiring form, m-calpain. Therefore, a few days after slaughter, only minimal amounts of detectable μ-calpain activity remain, but virtually all of m-calpain activity is present. Thus, by applying sufficient calcium to activate m-calpain, tenderness should be improved by its enzyme degrading action on certain muscle proteins. However, a particular protein known as calpastatin is present that inhibits calpain activity, but freezing meat causes the calpastatin that is present in muscle tissue to partially lose its ability to inhibit calpain. Therefore, it is reasonable to assume that if calpastatin activity can be decreased by freezing, freezing would enhance the effects of calcium application in activating the calpains. Thus, the objectives of this report were to determine 1) if calcium-containing marinades would improve tenderness, 2) if the activated calpain enzyme system could explain any improvement in tenderness, and 3) if prior freezing of meat would enhance the marinades' effect on tenderness.

Procedures

Data reported will be combined from three separate experiments designed to determine if calcium chloride marination would improve beef tenderness. Two rib eye steaks 1 inch thick were obtained from a total of 19 beef carcasses 5 days after slaughter. Half of the steaks were marinated in approximately 2.5 cups of an 150 mM calcium chloride solution for 48 hr at 39°F. Control, nonmarinated steaks were vacuum packaged and stored at 39°F for the same duration (48 hr). After each marination time, steaks were immediately cooked and tenderness was determined by measuring the force needed to sever a cooked meat core (1/2 inch). From 5 marinated and 5 nonmarinated steaks, samples were removed to determine the activities of the calpain enzyme system.

In another experiment to determine if freezing meat prior to calcium marination would enhance its effect, four rib eye steaks 1 inch thick were removed from 10 beef steer carcasses 6 days after slaughter. One steak from each animal was marinated 48 hr in 2.5 cups of 150 mM calcium chloride at 39°F. The other steak, which served as the nonmarinated control, was vacuum packaged and stored at 39°C for the same duration (48 hr). The other two steaks from each steer were frozen at -22°F for 6 wk after which steaks were thawed for 18 hr at 39°F. Thawed steaks were then treated as previously outlined for the fresh (nonfrozen) steaks. Each steak was cooked and tested for tenderness as in the previously mentioned experiment. Samples were also taken to measure the activity of calpastatin to see if its activity decreased with freezing and with marination.

Results

The calcium-containing marinades were successful in improving tenderness as revealed by the lower Warner-Bratzler shear values (Table 1). By applying calcium to the meat, the calcium concentrations were high enough to activate m-calpain which is not activated during normal aging of beef. After marination, the activity of both m-calpain and calpastatin decreased (Table 1). Therefore, tenderness was enhanced by m-calpain degrading certain muscle proteins that contribute to the tenderness of meat.

When fresh (nonfrozen) steaks were marinated, tenderness was improved (lower Warner-Bratzler shear values; Table 2). When steaks were frozen for 6 wk, thawed at 39°F, and aged for an additional 2 days without marination, there was a marginal improvement in tenderness revealed by a decrease in Warner-Bratzler shear force compared to the control fresh steaks. However, the greatest improvement in tenderness occurred when steaks were frozen, thawed, and calcium-marinated.

Calpastatin activity did decrease with freezing (Table 2) which may explain why calcium-marination was more effective after freezing than before freezing. With less active calpastatin, the calpain present would be inhibited less; therefore, calpain would be able to degrade more of the muscle proteins needed to improve tenderness.

In conclusion, calcium-marination of beef improves tenderness by activating naturally occurring muscle enzymes (calpains). This method of tenderizing meat is also enhanced by freezing. These are practices (freezing and marination) that normally occur in households and would serve as a form of calcium fortification and meat tenderization.

Table 1—Effects of calcium marination on Warner-Bratzler tenderness shear force and activities of m-calpain and calpastatin

Trait	Control	Marinated
Number of steaks	19	19
Warner-Bratzler shear, lb[a]	13.6	10.9
Number of samples	5	5
m-Calpain activity	1.5	.45
Calpastatin activity	.62	.13

[a] A higher shear force value indicates less tender meat.

Table 2—Effects of freezing and calcium marination on Warner-Bratzler tenderness shear force and calpastatin activity

	Fresh		Frozen	
Trait	Control	Marinated	Control	Marinated
Warner-Bratzler shear, lb[a]	9.57	7.85	8.8	5.96
Calpastatin activity	2.22	.81	1.85	.88

[a] A higher shear force value indicates less tender meat.

[1]Whipple is a research associate and Koohmaraie is the research leader, research physiologist, Meats Research Unit, MARC.
[2]The full reports of this work will be published in J. Anim. Sci. and Meat Sci.

The Effectiveness of Subjecting *Bos indicus* Crossbred Beef Carcasses to Higher Temperatures to Improve Tenderness

Georgianna Whipple, Mohammad Koohmaraie, Michael E. Dikeman, and John D. Crouse[1,2,3]

Introduction

Many studies have evaluated changes that occur in muscle during the aging process and how they relate to meat tenderness. Other research has shown that subjecting carcasses to higher temperatures soon after slaughter speeds the aging process that ultimately results in improved tenderness. Several things may explain this effect. The higher temperature causes the pH (acidity) of the muscle to decrease faster. Also, the combination of lower pH and higher temperature could promote an earlier release of calcium into the muscle, which normally occurs in muscle tissue after slaughter. This increase in calcium concentration in turn activates the calpain enzyme system (a naturally occurring enzyme system that is found in muscle tissue). When calpain is activated by calcium, it has the potential to degrade certain muscle proteins that must be degraded for meat to be tender. A discussion of this is found in the previous article. Therefore, because meat from *Bos indicus* breed crosses often is less tender than meat from *Bos taurus* breeds, we studied whether tenderness could be altered by carcass high-temperature conditioning and, if so, what mechanisms are involved.

Procedures

Seven heifers and four steers of 5/8 Sahiwal x Angus, Hereford or Angus x Hereford weighing an average of 986 lb were slaughtered at 15 to 17 mo of age. Carcasses were not electrically stimulated. Eleven carcass sides were high-temperature conditioned (HTC) at 72°F for 6 hr, then chilled at 30°F for 18 hr. The opposite control sides were chilled at 30°F for 24 hr. Muscle pH and temperature were monitored at 3 hr intervals for 12 hr, and recorded again at 24 hr. After 24 hr, the loin muscles were removed from both carcass sides. Steaks one inch thick were cut, vacuum-packaged, and aged at 39°F for 3, 7, and 14 days. Tenderness was determined on steaks 1 and 14 days post-slaughter by Warner-Bratzler shear force, which measures the amount of force required to sever a 1/2 inch cooked meat core. Also, cooked steak samples were evaluated by a trained sensory panel. The ease to which muscle fibers break (fragment) under controlled homogenization (known as muscle fiber fragmentation) also was measured at 1, 3, 7, and 14 days. To determine the activity of the calpain enzyme system, loin muscle samples were removed within 1 hr (0 hr), 6 hr, and 24 hr after slaughter. Loin muscle samples also were obtained to measure muscle cell length and the activity of other enzymes, known as cathepsins. However, no treatment differences were found for muscle cell length or cathepsin enzyme activity; therefore, those data will not be presented.

Results

As expected, muscle temperature remained higher in the HTC carcasses at 3, 6, 9, and 12 hr than in the control loin muscle (Figure 1). However by 24 hr, the HTC muscles had cooled to the temperature of the control muscles. Figure 2 indicates that the muscle pH of HTC sides was lower than the pH of control muscles at 6, 9, and 12 hr post-slaughter, indicating that the higher temperature hastened the rigor process.

High-temperature conditioning did prove successful in improving tenderness to a small degree in the *Bos indicus* carcasses; however, the sensory panel detected more of an off-flavor in the HTC steaks at day 14. Cooked loin steak samples from HTC sides were more tender as indicated by lower Warner-Bratzler shear force values than control steaks at 1 day post-slaughter (Table 1). However, sensory-panel scores failed to reveal this difference. At day 14, neither Warner-Bratzler shear force values nor sensory panel scores were significantly different, statistically. However, the muscle fiber fragmentation values, that indicate the amount of protein degraded which allows muscle fibers to fragment more easily, were greater at day 3, 7, and 14 in samples from HTC loin steaks than from control steaks. Therefore, it appears that the higher temperature treatment increased the rate that muscle proteins were degraded or, in other words, the tenderization process. However, by 14 days the control steaks had had enough time to decrease the amount of difference in tenderness.

Because differences in the rate of muscle protein degradation occurred, one would expect to find differences in the activity of the enzyme system responsible for these changes. Activities of the μ-calpain and calpastatin (a protein that inhibits calpain activity; see previous article) were determined at 0, 6, and 24 hr post-slaughter. Figure 3 reveals that μ-calpain declined more rapidly in HTC muscle than in control muscle. By 6 hr postmortem, 81 and 62% of the initial activity was lost in the HTC and control samples, respectively. At 24 hr, additional declines of 7% for HTC samples and 3% for control samples were observed. Therefore, control muscle had more activity remaining at 24 hr post-slaughter, which could indicate that less μ-calpain was utilized in degrading muscle proteins, because once μ-calpain is activated by sufficient calcium, it slowly loses its activity. Also, calpastatin loses activity in muscle tissue after slaughter; and high-temperature conditioning hastened its loss of activity. In the HTC samples, 35% activity was lost by 6 hr, whereas control muscles maintained almost all initial (0 hr) activity. Calpastatin activity remained higher in the control muscle at 24 hr.

Results from this study indicate that high-temperature conditioning marginally improved tenderness of loin steaks from 5/8 Sahiwal crosses and, of all the biological traits measured, only calpain and calpastatin activity were affected by this treatment. Therefore, μ-calpain and(or) calpastatin probably play a major role in tenderizing meat.

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[2]The full report of this work was published in J. Anim. Sci. 68:3654-3662, 1990.

[3]The authors would like to express their gratitude for the technical support of Sue Hauver, Kathy Mihm, Pat Tammen, and Kay Theer.

Table 1—Least-squares means for Warner-Bratzler shear values, sensory-panel scores and muscle fiber fragmentation values

Trait	High-temp. conditioned	Control
Day 1		
Warner-Bratzler shear, lb[a]	18.3	21.1
Sensory panel		
Tenderness[b]	3.7	3.6
Juiciness[c]	4.9	5.1
Off-flavor[d]	2.6	2.7
Day 14		
Warner-Bratzler shear, lb	15.2	16.9
Sensory panel		
Tenderness	4.5	4.4
Juiciness	5.1	4.8
Off-flavor	2.5	2.8
Muscle fiber fragmentation[e]		
Day 1	40	35
Day 3	45	32
Day 7	55	42
Day 14	65	55

[a] A higher shear force value indicates less tender meat.

[b] A score of 6=moderately tender,... 4=slightly tough.

[c] A score of 6=moderately juicy,... 4=slightly dry.

[d] A score of 4=none; 1=intense.

[e] A higher muscle fiber fragmentation value indicates that more protein has been degraded; thus, muscle fibers are easier to fragment.

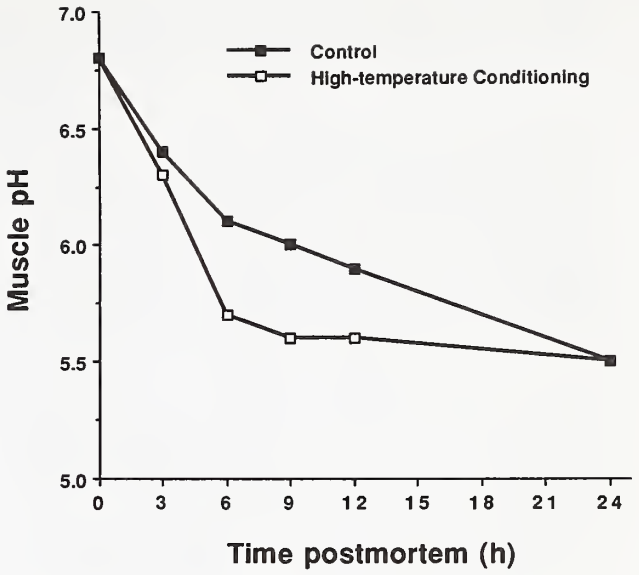

Figure 2 – Effect of high-temperature conditioning on pH of the loin muscle.

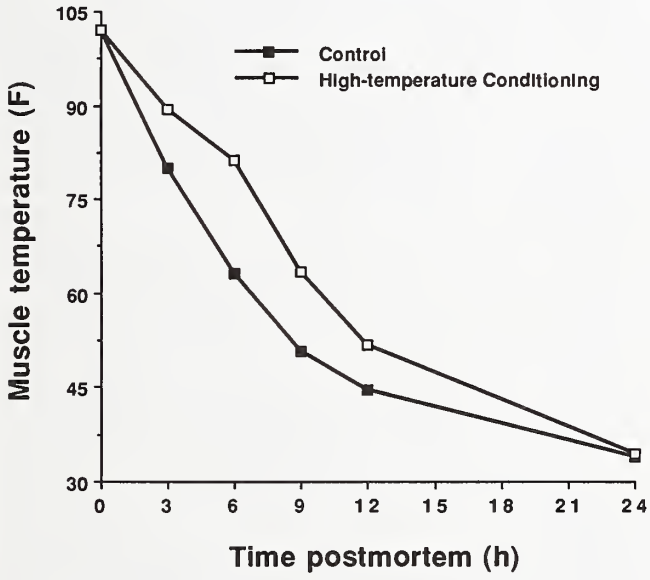

Figure 1 – Effect of high-temperature conditioning on temperature of the loin muscle.

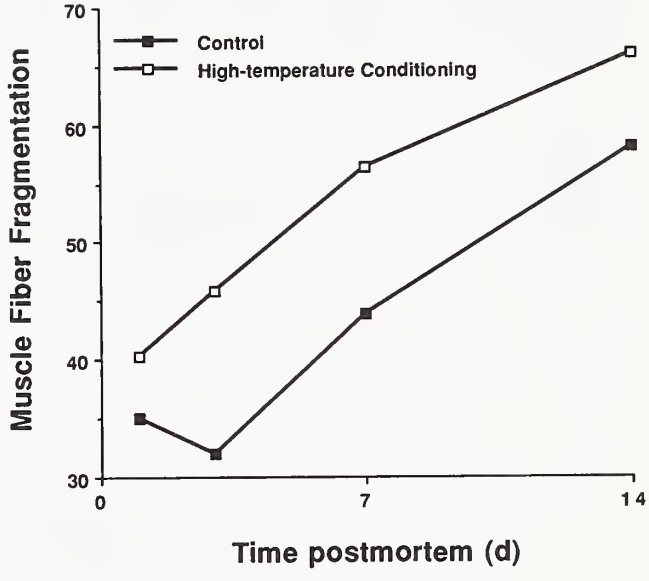

Figure 3 – Effect of high-temperature conditioning on loin muscle fiber fragmentation during postmortem storage.

Characterizing Stress in Feeder Cattle

G. LeRoy Hahn and John A. Nienaber[1,2,3]

Introduction

During the period August 6 to 10, 1992, a heat wave moved through central and eastern Nebraska. Maximum air temperatures were in the 90 to 95°F range, generally not considered to be extreme during the summer season. However, during this particular episode, the accompanying humidity was higher than normal (50 to 70% during the hottest portions of the day), with light to moderate winds except on August 8 when the wind was fairly strong. The relatively cool preceding summer weather had not adequately conditioned livestock to high levels of heat stress. As a result, several hundred feedlot cattle died in this area. Generally, animals most vulnerable to heat stress are new or recent arrivals in the feedlot, or those nearing market weight. Surviving cattle experience a reduced feed intake as a result of the heat stress which affects growth and efficiency.

The described heat wave is a vivid reminder: weather is a factor that cattle producers must deal with on a daily basis. Heat or extreme cold can reduce performance, health, and/or well-being. Those effects can be compounded by precipitation, wind, or poor nutrition in cold weather and high humidity in hot weather. An article, "Weather and Climate Effects on Beef Cattle," in the 1985 MARC Beef Research Progress Report No. 2 summarized some of the effects based on research observations, and discussed management alternatives for coping with adverse environments.

This report concerns measurement of stress in feeder cattle fed ad libitum. Objective characterization of stress is an essential element in determining the impact of environmental stressors, especially in establishing threshold limits (Figure 1) for reduced performance, health, or welfare. In passing, it is important to recognize that stress is an integral part of life. While usually considered a negative factor, it can also be a positive influence when it leads to coping and adaptation.

Blood hormone levels, such as cortisol, have typically served as stress response measures; however, blood sampling is an invasive technique which has limitations. We have recently investigated stress responses in terms of alterations in body temperature. In healthy animals, body temperature is an integration of heat-producing and heat-dissipating processes, and includes short- and long-term thermoregulatory responses to environmental stressors which ultimately affect animal performance. The focus of this report is on characterizing stress through analysis of the dynamics of body temperature fluctuations in feeder cattle. The results are used to examine stress thresholds.

Body temperatures reported here are represented by tympanic ("ear-drum") temperature requiring no surgery or other invasive procedures. Tympanic temperature has been shown in earlier research at MARC and elsewhere to be a sensitive measure of animal responses to environmental or disease-related challenges, as illustrated in Figure 2 for a cold-conditioned feedlot steer during a spring heat wave. Tympanic temperature represents the temperature of the hypothalamus, which plays a vital role in regulating endocrine and immune functions and is generally considered to have a central role in regulating feed intake.

Procedure

Tympanic temperatures were measured at 320-sec (1988) or 15-sec (1990) intervals in growing feeder cattle kept in nonstressing (cool) and heat-stressing (hot) environments at the MARC Environmental Laboratory. A silage-based diet was fed ad lib. A 2-wk cool period (50° + 12°F daily cyclic conditions) preceded each hot period. Each animal was exposed to several levels of daily cyclic hot conditions during successive treatment periods.

Various ways were evaluated to characterize the dynamics of the tympanic temperature responses to cool and hot conditions. The most successful method was a relatively new mathematical procedure called fractal analysis, which computes a fractal dimension as a measure of the "roughness" of the process analyzed. The method provides a value which objectively describes dynamic processes such as the animal's thermoregulatory system response to various environments.

Results

An example tympanic temperature record (15-sec measurement interval) is shown in Figure 3 for a steer during the last 2 days of exposure to nonstressing cool conditions and the first 6 days of exposure to heat. Some similarities are obvious in the overall response to hot conditions shown in Figure 3 for the laboratory steer and in Figure 2 for the feedlot steer. Similarities are especially strong for the diurnal range and pattern of body temperature rhythms and the daily declines in maximum and minimum body temperature during acclimation to heat.

Measurements obtained from the laboratory steer in controlled, repeatable daily cycles of temperature and humidity clarify the animal's response to the onset of hot conditions. Figure 3 shows the dynamics of the adaptive response and associated feed intake when the day-to-day variability of the outdoor environment is removed. The initial (acute) response to hot conditions requires about 3 days for the animal to regain a measure of balance between the metabolic heat production and the ability to dissipate heat. During this time, the lag between air temperature and body temperature is reduced; in the Figure 3 illustration, the lag reduced to about 3 to 4 hr after 3 days. Also after 3 days, maxima and minima for the tympanic temperature cycles steadily decreased during the acclimation phase, while feed intake tended to rebound somewhat. Measurements on all animals in the two experiments followed similar patterns, with the peaking of tympanic temperature typically occurring on the 3rd or 4th day after onset of heat. More extended datasets show that after about 7 or 8 days of exposure to heat, thermoregulation enters a chronic stage where the tympanic temperature cycles about a higher average value (depending on how hot the air temperature is) than during cool conditions. Feed intake is approximately an inverse situation, with the amount consumed at a reduced level in the chronic stage.

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[1]Hahn is the research leader, and Nienaber is an agricultural engineer, Biological Engineering Research Unit, MARC.

[2]This article is based on a published report in the Journal of Thermal Biology 17(2):115-120 (1992).

[3]Invaluable technical assistance was provided during this research by Roger Eigenberg, Dr. Yud-Ren Chen, Dr. Anne Parkhurst, and Dr. Tim McDonald. Technical support during the experiments by Lynn Gose, Eldon Shetler and Neal Kreutz, and the assistance of Elda Peterson in manuscript preparation, are also acknowledged.

The results in Figure 3 also illustrate the fine detail of thermoregulatory responses obtained from the 15-sec sampling interval, which shows the very distinct differences in tympanic temperature patterns for an animal exposed to nonstressing and stressing temperatures. The short-term dynamic variations of tympanic temperature about the underlying daily cycles are considerably "rougher" during cool conditions than during hot conditions which challenge the animal's thermoregulatory system.

The "roughness" of tympanic temperature responses to each condition was analyzed by computing fractal dimensions, as discussed in the Procedure section. Table 1 summarizes fractal dimension (D) results for available tympanic temperature data from three steers exposed to five temperatures in the 1988 test (320-sec recording intervals), and Table 2 provides averaged D-values and variances for six steers exposed to four temperatures in the 1990 test (15-sec recording intervals). Dataset A in Table 2 includes D-values computed from tympanic temperature records throughout the exposures to the various air temperatures, while Dataset B is limited to records during the chronic stages of exposure to each temperature. Results in these tables show that:

1. Fractal dimensions can be used to objectively classify thermoregulatory responses to cool and hot environments when based on tympanic temperatures recorded at intervals of 5 to 10 minutes.
2. Using tympanic temperature recording intervals of 15 sec does not provide distinctions among fractal dimensions for the various environments; other analyses show the effect to be true up to sampling intervals of 150 seconds.

The reason(s) for the apparent effect of sampling interval on fractal dimension are not yet clear, but may be related to short-term biological changes (such as vascular blood flow) or a limitation of the fractal technique. Given this possible limitation, the computation of fractal dimensions from tympanic temperatures recorded at intervals of 5 to 10 min provides a new approach to characterizing animal stress. The method provides results which are reasonably robust and repeatable across time and across animals. Further, the approach is particularly beneficial since it is based on a noninvasive measure which can be recorded without disturbance of normal animal routines and without human intervention.

Further research into practical application of fractal dimensions to characterize stress is planned in several areas. The first is to establish how well the laboratory-derived characterizations of stress responses can be related to field situations for a variety of environments. Initial observations from cattle in naturally varying cool and hot environments indicate computed fractal dimensions are consistent with those from laboratory data. A second area involves further evaluation of differences in fractal dimensions over time in the same animals (including the possibility of interacting stressors such as nutritional status). Observed differences among individual animals may provide a basis for genetic selection for tolerance to heat or other stressors. Evaluation of the approach to measure responses to other types of stressors (such as handling and transport) is a third area. A fourth area, the one which we initially targeted as a basis for this research, is further evaluation of threshold limits for stress as described in Figure 1. An example of using results of the fractal analysis for threshold definition is described in the next section.

Biological thresholds. Objective classification of stressors provides the basis for examining stress thresholds in growing animals. Using the information from Table 1 as an example, a plot of the fractal dimension, D, as a function of the average environmental temperature (Fig. 4) shows a threshold for reduced D values at about 77°F for growing cattle fed ad libitum. Values from Table 2 further support a threshold near 77°F. This indicates that for our experimental animals in thermal environments without exposure to strong radiant loads, the threshold limit for coping, above which performance is likely to be reduced, is about 77°F. It is interesting that these same experimental animals had a coincident feed intake decline threshold at 77°F. The association between tympanic temperature and feed intake thresholds is further strengthened by subsequent research we have done showing a strong linkage between feeding events and tympanic temperature.

Summary

Analyses of tympanic temperature data obtained as a measure of thermoregulatory function in feeder cattle fed ad-libitum indicate that responses to thermoneutral and several levels of heat-stressing environments can be objectively characterized by computed fractal dimensions. Variations in fractal dimensions resulting from thermal environment influences were greater than variations among animals.

There are apparently some biologically based limitations on sampling interval frequency for tympanic temperature as a basis for computed fractal dimensions. Intervals shorter than 2 1/2 min are generally unacceptable for characterizing responses, while intervals between 5 and 12 1/2 min are acceptably definitive. Using a 5 1/3 min sampling interval, a clearly defined fractal threshold was observed at about 77°F, indicating thermoregulatory stress above that temperature. This threshold is coincident with a threshold for feed intake decline, strengthening a previously noted linkage between thermoregulatory function and feed intake.

Other potential benefits of the fractal analysis technique include evaluating thermoregulatory responses to other types of stressors, and for estimating heat tolerance of animals as a basis for genetic selection to improve performance, health, and well-being of livestock in hot climates.

Table 1 – Fractal dimensions of the tympanic temperature of steers in nonstressing and stressing environmental temperatures—from 1988 data recorded at 320-sec intervals

Environmental temperature (°F)	Computed fractal dimension			
	Animal ID 3382	Animal ID 3456	Animal ID 3472	Average
50 ± 12	1.76	1.78	1.77	1.77
79 ± 12	1.74	1.66	N/A	1.70
82 ± 12	1.64	1.42	N/A	1.53
86 ± 12	1.61	1.45	1.34	1.47
93 ± 12	1.28	N/A	N/A	1.28

N/A = not available

Table 2 – Fractal dimensions of the tympanic temperatures of steers in nonstressing and stressing environmental temperatures-from 1990 data recorded at 15-sec intervals

Environmental temperature (°F)	Computed fractal dimension ± standard deviation		
	Dataset A		Dataset B
	Based on all 15-sec interval data pts in daily record	Based on sampling 15-sec dataset every 20th point (300-sec intervals)	Based on sampling 15-sec dataset every 40th point (600-sec intervals)
50 ± 12	1.73 (24)*	1.78 (20)	1.77 (42)
86 ± 12	1.69 (16)	1.69 (14)	1.55 (28)
90 ± 12	1.69 (16)	1.51 (16)	1.37 (28)
93 ± 12	1.72 (15)	1.44 (13)	1.35 (28)

*Parenthetical numbers are the steer-days of record used from the 6 steers in the experiment.

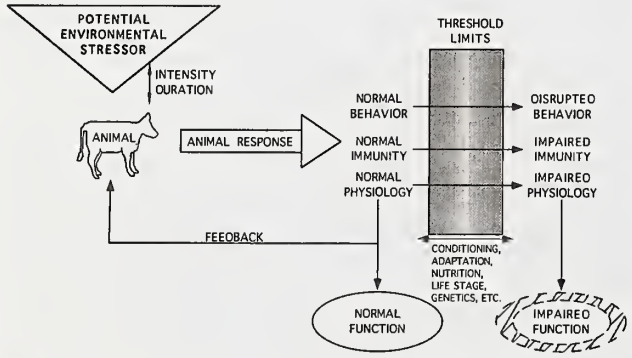


Figure 1—Responses of animals to potential environmental stressors which can influence performance and health.

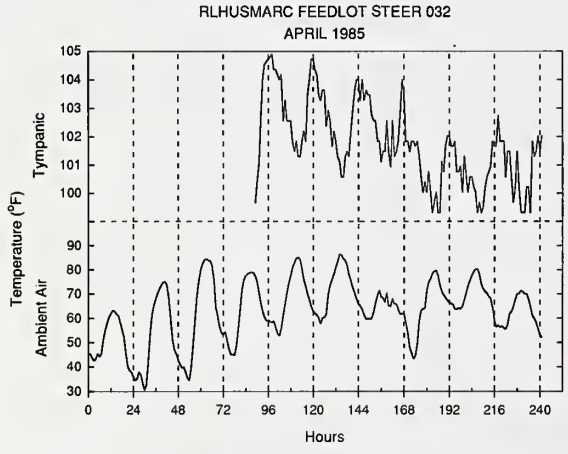


Figure 2—Tympanic temperatures recorded from a MARC feedlot steer for several days during a spring heat wave (records are at hourly intervals, with Hour 0 = midnight). Air temperatures for the period preceding the tympanic temperature record are provided to show the progression of the heat wave. Noteworthy points are 1) the hyperthermic (high) body temperatures recorded in an animal conditioned to cold temperatures; 2) the daily declines in maximum and minimum body temperature as the animal acclimates to the heat; 3) the return to normal cycles and ranges of body temperature as the heat wave abates; and 4) the 6 to 12 hr lag time between maximum and minimum air temperature and the subsequent body temperature, with maximum body temperatures occurring near midnight.

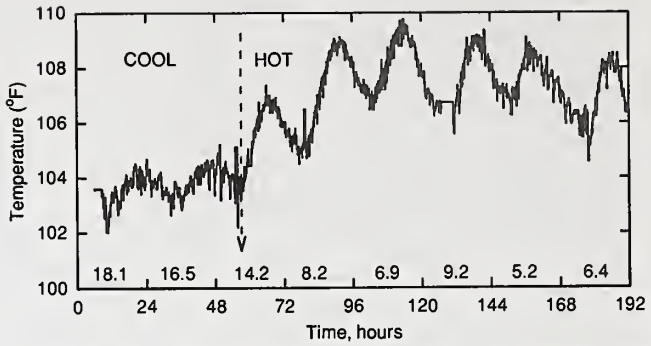


Figure 3—Tympanic temperatures recorded over several days from a steer exposed to moderate (50° ± 12°F) and hot (93° ± 12°F) environments. The arrow indicates the time at which the hot conditions were imposed. Midnight of each day occurred at the listed 24-h multiples. Daily feed intakes (lb) associated with each day are included.

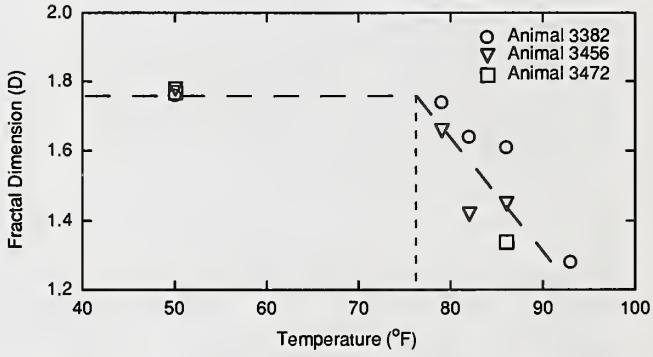


Figure 4—Plot of fractal dimension D (computed from tympanic temperatures of steers in cool and hot environments) as a function of environmental temperature. The intersection of lines for animals in stressed and unstressed states occurs near 77°F, indicating the threshold for onset of stress to be about that temperature.

Heat and Moisture Production and Dissipation in Beef Cattle

John A. Nienaber, G. LeRoy Hahn, and Anders Ehrlemark[1]

Introduction

Calorimetry or the measurement of heat transfer between animals and the environment has been conducted at MARC for several years. The primary objective of calorimetry has been the evaluation of maintenance energy requirements of animals. For cattle, maintenance requirements for either lactation or growth have been of interest.

Calorimetry can also provide information useful in evaluating heat dissipation by animals in various environments. Recent measurements were completed at MARC by a Swedish engineer to provide answers to a beef housing problem. Current information has proven to be inadequate for the design of beef housing ventilation systems, resulting in unacceptably high humidities in the buildings. Therefore, a study was designed to measure the heat and moisture production of cattle in response to air temperatures from 43 to 75°F.

Procedure

Four crossbred steers (Hereford x MARC III) weighing 575 to 862 lb were housed in environmental chambers for a 4-wk period to simulate housing conditions in a typical cold or temperate climate. The animals had free access to feed and water and were fed a silage based ration containing 59% dry matter, a metabolizable energy content of 1.37 MCal/lb, and 10.6% protein. The animals were adjusted to the ration and the facilities in a previous experiment. The animals were weighed at the beginning and the end of the experiment.

Two controlled-environment chambers with two stalls each were operated at four temperatures (43, 54, 64, and 75°F). One chamber was set at 75°F the first week and then lowered in weekly steps until it was 43°F the fourth week, while the second chamber was started at 43°F and then raised in the same manner to 75°F. The temperatures of the controlled environment test-chambers were changed in the first day of each measurement week. Body temperatures were recorded continuously, while heat production and heat dissipation measurements were made during the last two days of the week.

Total heat production of each animal was measured during a 23-hr period with a headbox which held both feed and water. There was one headbox in each chamber, so data were collected from two animals (one in each chamber) simultaneously. The equipment was moved from pen to pen when needed and removed when not in use.

Heat dissipation by sensible and latent (moisture evaporation) means was measured during three 3-hr periods per measurement day. Sensible heat loss (radiant and convective) could not be measured directly, but local values were calculated from spot measurements of animal and surroundings (wall and pen) surface temperatures and airflow. Radiant heat loss was calculated from surface temperatures measured with a calibrated infrared thermometer. Convective heat loss was calculated from air temperatures, animal surface temperatures, and air movement as measured around the animal. Convective heat loss was also dependent on the heat conductivity of the animal hide as affected by hair length and body fat content.

Latent heat loss included moisture loss from respiration and from the skin surface. Moisture loss from the skin surface was measured for about one hour on each of three sites on each animal during each heat dissipation measurement. Skin surface moisture loss was measured with a ventilated capsule designed to give the same heat and vapor diffusion resistance as the air film around the animal. The amount of water vapor collected by the capsule was determined from the standard corrected air flow through the capsule, and the calculated difference in moisture content between air entering and leaving the capsule.

The headbox provided the means to collect all respiration and head/neck skin moisture losses. Like the ventilated capsule, moisture loss was based on the standard corrected air flow through the headbox, and the calculated difference in moisture content between air entering and leaving the headbox.

Results

Time of day had minimal effect on heat loss which averaged .648, .717 and .711 Kcal/hr/lb for time periods of 04:30 to 7:30 a.m.; 10:30 a.m. to 1:30 p.m.; and 5:30 to 8:30 p.m., respectively. Average total heat loss affected by temperature and the partition of total heat loss into sensible, cutaneous, and respiratory heat loss are presented in Figure 1. Total heat loss increased by only 12% as temperatures dropped from 74 to 43°F. Latent heat loss, shown as respiratory and cutaneous heat loss, increased 2.5 fold from 43 to 75°F. Latent heat loss increased from 15% to 45% of the total heat loss from 43 to 75°F, and must increase to 100% of the total heat loss when air temperature equals or exceeds body temperature at 101.5°F. At that temperature, sensible heat loss would become negligible since there would be no temperature gradient between the animal and its environment. Over the temperature range studied here, nearly all of the increase in moisture loss resulted from increased cutaneous heat loss (increased from 9 to 32% of the total heat loss). Respiratory heat loss increased from 6 to 12% of the total heat loss.

It is apparent that moisture loads on a ventilation system become increasingly important as temperature increases. Although warm air physically holds a greater amount of moisture, and adding heat effectively removes moisture, these data show that the increased moisture production of beef animals could cause increased ventilation requirements without conserving animal heat loss. It is also evident that housing beef animals requires close attention to ventilation. Heat stress occurs when an animal is unable to dissipate its body heat through sensible and latent losses. The primary response of animals to heat stress, which may result from inadequate ventilation, is reduction in feed intake and subsequent growth. This is a primary factor in the problems which have limited the success of beef housing. Similar problems may occur whenever beef animals are held in close confinement, such as during transportation or marketing. It is vitally important that the producer, marketing manager, and the packer closely observe beef animals for signs of heat stress, even in relatively moderate or cool conditions. Skin wetness is an early sign that a beef animal

[1]Nienaber is an agricultural engineer, and Hahn is the research leader, Biological Engineering Research Unit, MARC; Ehrlemark is an agricultural engineer, Swedish University of Agricultural Sciences, Uppsala, Sweden.

is shifting its heat loss to latent means in order to offset heat stress. Increased activity associated with handling will increase the heat load and may have negative effects. Gentle handling, which is always important in moving animals, is even more critical when heat stress is evident.

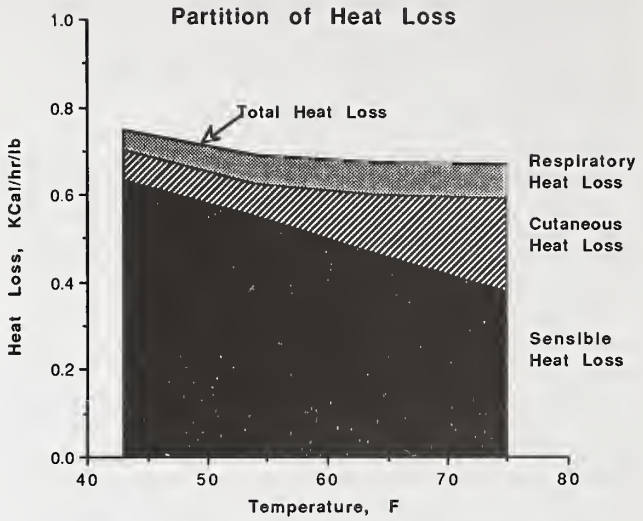


Figure 1 – Partitioned heat loss of crossbred steers averaging 720 lb and fed at room temperatures of 43°F to 75°F.

Influence of Controlled Energy Intake on Body Composition of Beef Steers

John W. Keele, Calvin L. Ferrell, Ralph N. Arnold, Michael E. Dikeman, and Melvin C. Hunt[1]

Introduction

Decreasing the amount of fat in beef decreases loss due to trim and the number of calories in a serving. Differences in fat content of cattle are caused by differences in genotype, gender, chemical agents and energy intake relative to functional requirements. An increase in energy intake increases both fat content and body weight. Based on this fact, differences in energy intake cause an association between fat content and body weight. This fact has led some scientists to suggest the hypothesis that fat content of animals of similar genotype and gender can be predicted from body weight even if there are differences in energy intake. In order for this hypothesis to be true, rate of gain for fat must be proportional to rate of gain for body weight. Previous research has demonstrated that this is not the case. Increasing energy intake decreases the days required to reach a constant slaughter weight and increases the fat content of cattle slaughtered at a constant weight. Hence, increased fat content is associated with fewer days required to reach the same final weight when genetically similar cattle consume different amounts of energy.

The contents of the gastrointestinal tract (gut fill) influences body weight. Gut fill varies among measurements of different animals or among repeated measurements of the same animal. For this reason, carcass weight is a better indicator of an animal's "true weight" than body weight.

The objectives of this study were to estimate differences in fat content caused by variation in energy intake and to determine the extent to which these differences are associated with carcass weight or days to slaughter.

Procedure

A total of 161 steers were used in the study. Steers were one of two biological types, 1) a small biological type which consisted of two-way crosses of Red Poll or Angus sires and Angus or Hereford dams or 2) a large biological type which consisted of crosses of Brown Swiss sires with two-way crosses of Angus, Hereford, Simmental, Limousin, and Charolais dams. Average initial weights of the small and large biological type steers were 588 and 696 lb, respectively.

Steers were fed a diet composed of 74% corn grain or a diet composed of 74% corn silage. Steers given the corn silage diet were fed ad libitum or fed to maintain body weight. Steers given the corn grain diet were fed ad libitum, fed a restricted amount of food so they grew at the same rate as steers given ad libitum corn silage, or fed to maintain body weight. Planned growth patterns of the steers for body weight are presented in Figure 1. Steers were slaughtered when they achieved one of four slaughter weight groups (Figure 1). Chemical composition for protein, water, fat and ash was determined for boned-out soft tissue near the 9th, 10th, and 11th ribs.

Results

Average body weights at slaughter were similar to those planned (Table 1). Differences in average hot carcass weight were associated with differences in average body weight at slaughter (Table 1).

Average chemical composition of the boned-out soft tissue near the 9th, 10th, and 11th ribs is presented in Tables 2 and 3. One of the main purposes of the study was to determine if all of the differences in fat content caused by changing energy intake were associated with differences in hot carcass weight or if some of these differences could be associated with time needed to reach the same hot carcass weight. Over half (55%) of the variation in fat content among steers of the same biological type was associated with hot carcass weight. An additional 10% of the variation in fat content was associated with days needed to reach the same hot carcass weight. Diet accounted for a further 10% of the variation in fat. Percentages of variation for protein, water, and ash attributed to weight, days, and diet were similar to those for fat.

How large were differences in chemical composition associated with hot carcass weight and days to reach the same hot carcass weight? For every 100 lb increase in hot carcass weight, there was an associated increase of 5.1% fat and a decrease of 1.0% protein, 4.1% water, and .06% ash of the soft tissues near the 9th through 11th ribs. For each additional 100 days that it took steers consuming ad lib silage or restricted grain to reach the same hot carcass weight as steers fed ad lib grain, their fat decreased 2.5%, protein increased .4%, water increased 1.9%, and ash increased .02%. These relationships should not be used to compare 14 month-old steers weighing 800 lb to 5 year-old steers of the same weight and genotype. However, these relationships should be valid over the normal range of growing and finishing periods because this time span is unlikely to exceed 600 days.

There were some nutritional treatments where the data did not fit the general patterns described above. Steers fed restricted grain in the third slaughter group contained less fat and more protein, water, and ash than the values predicted from hot carcass weight and days required to reach the same hot carcass weight. Conversely, steers fed ad lib grain in the fourth slaughter group contained more fat and less protein, water, and ash than the values predicted from hot carcass weight and days required to reach that weight.

We conclude from these results that that fat content decreases as dietary energy intake is reduced and the days required to reach a given hot carcass weight increase. However, nutritional manipulation can sometimes cause differences in chemical composition among similar steers that are not predictable from hot carcass weight or days on feed. For producers that receive a premium price for low-calorie beef, both energy consumption and slaughter weight can be manipulated to control the fatness of cattle. One of the criticisms of feeding cattle less energy to produce a leaner product is that it takes longer to reach slaughter weight and, hence, requires more feed. Recent research indicates that this may not always be the case. Economical production of lean beef seems possible.

[1]Keele is a research animal scientist, MARC; Ferrell is the research leader, Nutrition Research Unit, MARC; Arnold is self employed; Dikeman is a professor of meat science, Department of Animal Sciences and Industry, Kansas State University, Manhattan; Hunt is a professor of animal science, Kansas State University.

Table 1—Average hot carcass weight and slaughter body weight

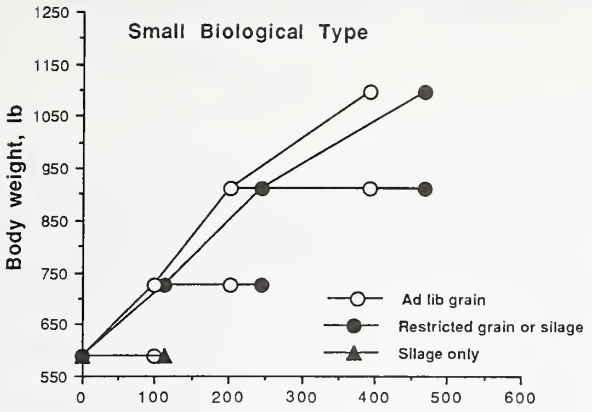
Level	Diet	Slaughter group	Hot carcass weight				Slaughter body weight			
			Small		Large		Small		Large	
			grow	maintain	grow	maintain	grow	maintain	grow	maintain
	grain	1		341		394		588		678
	forage	1		370		432		626		733
Ad lib	grain	2	485	504	579	579	822	846	967	927
Restricted	grain	2	425	489	502	518	700	811	844	883
Ad lib	forage	2	416	498	509	544	729	822	874	989
Ad lib	grain	3	579	500	696	590	956	835	1141	980
Restricted	grain	3	570	544	619	584	903	965	1040	1062
Ad lib	forage	3	553	507	615	509	954	943	1054	1009
Ad lib	grain	4	742		835		1216		1385	
Restricted	grain	4	769		879		1253		1370	
Ad lib	forage	4	747		837		1264		1465	

Table 2—Mean chemical composition(%) of soft tissue near 9th through 11th ribs of small biological type steers

Diet	Fat		Protein		Water		Ash	
	grow	maintain	grow	maintain	grow	maintain	grow	maintain
Slaughter weight group 1								
Grain		20.7		17.0		61.7		.87
Forage		24.5		16.2		58.7		.80
Slaughter weight group 2								
Ad lib grain	36.5	34.4	14.0	14.8	49.3	50.7	.70	.73
Restricted grain	31.9	31.8	15.3	14.7	52.7	53.5	.79	.75
Ad lib forage	29.0	29.1	15.7	15.1	55.5	55.3	.80	.78
Slaughter weight group 3								
Ad lib grain	34.4	26.0	14.7	16.1	50.6	57.2	.75	.83
Restricted grain	29.0	27.1	15.8	15.7	55.1	56.0	.79	.79
Ad lib forage	37.8	21.3	14.2	16.7	48.1	61.0	.72	.83
Slaughter weight group 4								
Ad lib grain	50.0		11.5		38.6		.57	
Restricted grain	46.7		11.4		41.2		.58	
Ad lib forage	45.7		12.1		41.4		.58	

Table 3—Mean chemical composiition (%) of soft tissue near 9th through 11th ribs of large biological type steers

Diet	Fat		Protein		Water		Ash	
	grow	maintain	grow	maintain	grow	maintain	grow	maintain
Slaughter weight group 1								
Grain		12.5		18.9		68.2	1.00	
Forage		18.6		17.6		62.7		1.85
Slaughter weight group 2								
Ad lib grain	29.5	24.8	15.9	16.7	54.4	58.4	.74	.84
Restricted grain	20.1	21.6	17.2	17.4	62.0	61.1	.85	.95
Ad lib forage	24.0	23.6	16.8	17.2	58.8	59.2	.82	.89
Slaughter weight group 3								
Ad lib grain	31.6	20.3	15.4	17.8	52.6	61.6	.78	.82
Restricted grain	22.7	16.3	17.6	17.9	59.4	65.0	.88	.83
Ad lib forage	27.2	12.1	16.5	19.1	55.9	68.1	.80	.96
Slaughter weight group 4								
Ad lib grain	42.1		13.3		44.7		.59	
Restricted grain	32.0		4.6		52.5		.70	
Ad lib forage	36.7		4.0		48.4		.69	



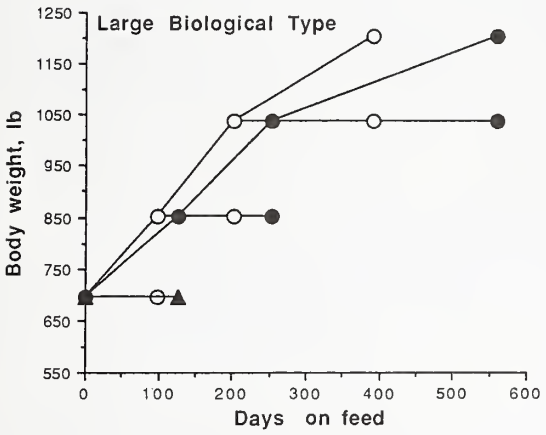


Figure 1—Planned growth patterns.

Management Factors Influencing the Feeding of Young Bulls for Market-Ready Beef

Michael D. MacNeil, Keith E. Gregory, and J. Joe Ford[1]

Introduction

Feed is a major part of the total cost in raising cattle from weaning to market-ready weights. Young bulls convert energy and protein from feeds to lean beef more efficiently than steers. Most consumers would prefer beef with less fat outside the muscle of retail cuts. Carcasses from bulls killed at about 17 months of age and weighing 1,300 to 1,450 pounds have less backfat; less kidney, pelvic, and heart fat; and yield more weight of closely trimmed retail product than carcasses of similarly managed steers. However, at this age carcasses of bulls also have less fat within the muscle and may be less tender. Production of beef from young bulls is a common practice in Europe. However, it remains a largely unused system in the United States. Aggressive and homosexual behaviors of bulls may explain part of the reluctance by U.S. feedlot operators toward feeding bull calves. In these studies we investigated factors under managerial control that might reduce undesirable behavior of young bulls and improve their performance.

Procedure

We conducted three experiments feeding 12 to 14 mo old bulls to marketweight. The feeding period lasted either 56 or 112 days. Factors evaluated were: group size (30-34 vs 60-68 head/pen), mixing bulls from different pens, and tranquilizing bulls before mixing them. Rations contained 1.2 to 1.3 Mcal of metabolizable energy per lb and 12 to 13% crude protein. Each pen of bulls was transported to a packing plant as a group and killed immediately after arrival. Carcass data were recorded 24 hr after slaughter.

Results

Bulls penned in smaller groups grew about 17% faster (2.7 vs 2.3 lb/day) than bulls penned in larger groups. However, feed consumed per day did not differ by group size. Bulls penned in smaller groups also had 17% more backfat (.38 vs .33 in) than their counterparts penned in larger groups. Based on these results, the optimal number of bulls per pen is less than 60. However, finding that optimum requires further research.

Tranquilizing bulls before mixing them reduced butting and riding immediately after that. However, as the tranquilizer wore off butting and riding increased. Over a 3 day period after mixing the bulls, no differences existed between tranquilized and nontranquilized groups in numbers of head butts or mounts. Tranquilized and nontranquilized bulls were similar in all performance and carcass characteristics measured.

Bulls reared from weaning with the same pen-mates can establish a "pecking order" at younger ages and less violently than bulls mixed at 1 yr of age. In comparison with bulls mixed at 1 yr of age, keeping pens intact from weaning had no effect on their growth, feed intake, or carcass attributes. We speculate that the few days needed to establish a "pecking order" in relation to the length of the feeding period offset this treatment effect.

[1]MacNeil is a research geneticist, Fort Keogh Livestock and Range Research Laboratory, Miles City, MT (formerly research animal scientist, Production Systems Research Unit, MARC); Gregory is a research geneticist, Genetics and Breeding Research Unit, MARC; Ford is a research animal physiologist, Reproduction Research Unit, MARC.

Evaluation of Four Computer Models for Prediction of Growth and Body Composition

Gary L. Bennett and Ralph N. Arnold[1,2]

Introduction

Leaner, high quality beef can be produced by making good management and genetic decisions. The problem is knowing what is a good decision. Computer models can be used to predict the outcomes of different ways of producing beef. Managers can choose their best system using these predictions combined with their financial and feed resource information.

Several computer models predict growth and body composition as part of an overall evaluation of beef production. Other models predict only growth and body composition. These models predict one or more of the following biological processes: the amount of feed consumed, the partition of consumed feed into nutrients for maintenance and growth, and the partition of nutrients used for growth into fat, lean, and bone.

This research compared growth and body composition prediction from four computer models. Standard situations and experimental results were used for the comparison. The goal was to decide whether any of the models were accurate enough to aid cattle producers who want to increase the leanness of beef. Another goal was to find ways to improve predictions.

Procedure

Three computer models of growth and body composition were extracted from models of overall beef production systems. The developers of these models emphasized feed intake and growth more than body composition. The fourth model evaluated was developed to predict growth and composition when feed intake was known. The four models were then used to make comparisons.

The standard situations compared were lean growth unrestricted by feed intake, forage diet, grain diet, compensatory growth, and medium and large size steers. Feed intake of forage and grain diets was determined several ways, i.e., using model predictions, using the same intake for all models, and as a percentage of body weight.

Three experiments were identified that had both feed intake and body composition available for comparison with model predictions. The experimental treatments included level of feed intake, type of feed, breed, age, and sex. Both actual feed intake and predicted feed intake were used for some comparisons.

Results

The computer models required either direct input of mature wt or other indirect input values that resulted in a mature weight. Direct or indirect input values for mature wt were adjusted so that protein growth rates were the same for the first 900 days following birth assuming growth was not restricted by feed intake. Fat growth rates were similar for all models until about 500 days and then diverged as animals approached maturity.

The four models responded differently to different levels of assumed feed intake. Models also differed when all-grain diets were compared with all-forage diets. Simulated body composition varied with level of feed in three models but only after severe restriction in another model. Two models simulated slight compensatory growth. The predicted effect of 200 days of restricted growth followed by ad lib intake ranged from 0 to 5% body fat at slaughter weight.

Differences among model predictions stemmed from assumptions about feed intake, maintenance requirements, protein:water ratios, and the partition of growth among different tissues. These were the result of differences in the interpretation of the growth process. Equalizing feed intake reduced differences in growth and composition when grain was fed but not when poor quality roughage was fed.

It was apparent from the simulation of standard situations that the evaluation of a beef production system will depend on the computer model chosen, especially if carcass composition is important. Comparisons with experimental results were done to find which situations were accurately predicted by the computer models.

Many predicted and experimental wt differed by more than would be expected by chance. Differences expressed as percentages of their experimental values were generally less for body wt than for fat, water, and protein weight. The accuracy of predicting fat was usually less than protein and water.

Predicted and experimental feed intakes for ad lib treatments were also different in many cases. There was a tendency to over- or underpredict intake for all treatments in an experiment, but this was not always the case.

A consistent pattern of differences, such as finding differences only in one type of cattle or for one kind of feed, was not apparent. This limited conclusions about how to improve the models. Weight gain was more accurately predicted than the composition of the gain. This suggests that more research is needed to determine the partition of gain to fat, lean, and bone. One conclusion reached was that when fat was considered to result from the storage of excess energy, then all errors in predicting feed intake and its utilization for maintenance and growth end up as differences in fat.

These comparisons suggested that other approaches to predicting the effects of nutrition on body composition need to be tried. To be useful in designing and evaluating systems of producing leaner beef, these approaches need to have fewer places where errors can occur or distribute errors more evenly among lean, fat, and bone.

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[1]Bennett is the research leader and Arnold was a research affiliate, Production Systems Research Unit, MARC.

[2]The full report of this work was published in Agricultural Systems 35:401-432 and 36:17-41, 1991.

Conversion Efficiency Through Weaning of Nine Breeds of Cattle

Thomas G. Jenkins and Calvin L. Ferrell[1]

Introduction

Beef cattle production entails the conversion of plant resources not normally considered as part of the food chain for humans into a food resource that partially fulfills human dietary needs. Traditionally, the beef industry has been segregated into production components, each having its own marketing endpoint. The cow/calf component of the industry produces progeny for introduction into the food chain conversion process. Energy and protein requirements of the commercial cow herd should be fulfilled as much as possible through direct harvest of forages by the animals. Within the U.S., a wide range of forage production environments exist.

Commercial producers have the flexibility to identify breeds or breed crosses to be used as producing females and to identify sire breed or breed crosses to mate with these cows. Previous research at MARC has demonstrated variation among and within breeds for traits affecting weight of calf produced at weaning. Cows representative of breeds with greater genetic potential for growth and lactation yield have been shown to produce calves that are heavier at weaning. Additional research at MARC has documented a positive relationship between genetic potential for production and energy requirement to maintain body weight of the cow. Differences in energy required to sustain the producing female suggest that breeds or breed crosses can be identified that are more effective in the conversion of forage resources into amarketable product. Earlier work conducted at MARC indicated that breed crosses more moderate in growth potential and lactation yield, were more effective in preweaning weight production of calves. The objective of the study was to determine if differences exist among breeds of beef cattle in the efficiency of converting food energy to weight of calf at weaning.

Procedures

In 1986, 16 pregnant multiparous cows from Angus, Braunvieh, Charolais, Gelbvieh, Hereford, Limousin, Red Poll, Pinzgauer, and Simmental cows that were 5 yr or older were assigned to the study. Four cows within each breed were assigned to one of four energy availability levels: 130, 170, 210 or 250 Kcal of metabolizable energy (ME) per metabolic body size ($wt.^{75}$) during nonlactating periods or during lactation fed at the rate of 170, 210, 240, or 290 Kcal ME/$wt.^{75}$. Individual animals remained at the assigned levels throughout the test period. Daily feed allotments of individual cows (Table 1) were based on the weight of the cow (measured approximately at the seventh month of gestation) at the time of the cow's assignment to the study. Cows were individually fed and received their daily allotment in a single feeding. Feed refused by the cows over a seven day period was measured and recorded. Feed consumed by the cow was determined as the difference between the feed provided for a seven day period minus the feed refusal. In mid-March each year all pregnant cows were transported to drylots for calving. Male calves were castrated at birth. Birth weights were recorded for all calves. Cow/calf pairs were returned to the test facility approximately 10-14 days after calving. Upon return to the test facility, lactating cows' feed allotments were increased.

Cows were exposed to sires identified within their respective breeds for a 90 day period beginning in mid-June of each year. During the breeding season, cows and calves were separated at approximately 4:00 p.m. daily, the cows were penned by breed, and cows remained in these pens until approximately 7:00 a.m. The 1987 calf crop was weaned in a single group at approximately 200 days. Within the two remaining production years, calves were weaned in two groups with average weaning age and range in age similar to 1987. Following weaning of the calves, daily feed allotments of individual cows were reduced to nonlactation levels.

Weekly feed consumptions for individual cows were summed for the three year test period. Individual calf records were used to adjust the weaning weights of calves to 200 days weaning age. Records of individual cows were summed. Biological efficiency is defined as the ratio of weight of calf weaned relative to the feed consumed by cows weaning calves. The efficiency ratio is an index of the effectiveness of converting feed resource to a marketable product. As used in the present evaluation, it is a measure of that amount of feed energy that was consumed that is available for use by the cow to produce a product. For cows weaning calves, total feed consumption, sum of calf weights weaned, and the ratio of biological efficiency were analyzed to evaluate the effects of breed, level of energy availability, and the breed by level of energy availability upon these traits.

Results

For the traits of interest, the interaction of breed by energy availability was not found to be a significant source of variation. This indicates that the rank among the breeds for these traits would be expected to be the same across all four energy availability levels. Both breed and level of energy availability affected total feed consumption, average weaning weight, total weaning weight for the three year period, and biological efficiency ratio.

Estimates for the traits of interest by level of energy availability are reported in Table 2. Productivity and total weight weaned for the 3 yr period increased as level of energy available to the cow increased. Over the test period, cows receiving the highest feed level produced 30% more weight at weaning than did cows fed at the lowest intake level but only 8% more than at the other two levels. Input, feed consumed during the 3 yr period, was 78% greater for cows receiving the highest feed level, and 65% and 39% for the intermediate groups relative to the feed consumed by the cows assigned to the low feed level. Although cows receiving the lowest quantity of feed produced the lowest product yield for the test period, this group of cattle were 27% more effective in converting feed energy consumed to calf weight than the two highest feed available levels and were 7% more efficient than the 170 kcal/$wt.^{75}$ group.

Breeds of cattle previously characterized as having higher genetic potential for growth tended to be of higher rank for this output component. Comparison of weaning weight yield for the 3 yr period among the breeds indicates some reranking among the breeds for output (Table 3).

[1]Jenkins is a research animal scientist, Production Systems Research Unit; and Ferrell is the research leader, Nutrition Research Unit, MARC.

Braunvieh and Charolais had the greatest yield and Hereford the lowest. Input information for the purpose of this report represents feed consumed by cows weaning calves. Charolais, as a breed group, consumed the least amount of feed and Braunvieh and Angus consumed the most. Among straightbred cows producing calves of the same breed, two separate groups could be identified: Red Poll, Braunvieh, Limousin, Pinzgauer, Charolais, and Gelbvieh were the most effective in converting feed energy resources to a marketable product (Table 3). Hereford, Angus and Simmental breeds were less effective in converting the energy resource to weaning weights. Among all breeds, approximately 16% difference was observed between the most and least efficient breeds.

These results indicate that differences in the effectiveness of the conversion of food energy resources to marketable product may be affected by level of food energy availability and choice of breeds. From a feed energy standpoint, the producer needs to be aware of the productivity potential of the forage resources available and the desired level of productivity sought for the cow herd. Harvested energy resources tend in general to have higher cost associated with them. Efforts of the cow/calf producer to improve or maximize total weight weaned through use of supplemental feeding programs or through intentional understocking may result in less than optimum production.

When compared on the basis of pounds of calf weaned per unit of food energy consumed by the producing cow, differences exist among the nine beef breeds evaluated in this study. These breeds have been previously characterized with regard to growth potential and ability for milk production. Using productivity information in conjunction with measures of average production efficiencies for breeds should enable a producer to identify a mating system and the breeds of cattle compatible with the mating system for a defined production environment.

Table 1— Composition of diets (percent of dry matter)

Ground alfalfa	77.5	
Corn	17.5	
Corn silage	5.0	
Metabolizable energy	1.03	Mcal/lb
Crude protein	16%	

Table 2--Effect of level of energy availability upon measures of output and input over three years

	Energy availability (Kcal/wt.^{75})			
	130	170	210	250
Total feed consumed,(Mcal)	14,391	19,701	23,776	25,739
Three year total weaning wt, lb	873	1,126	1,193	1,237
Efficiency, (lb/Mcal)	.061	.057	.048	.048

Table 3—Effect of breed upon measures of output and input over three years

	Total feed consumed (Mcal)	Three year total weaning wt (lb)	Efficiency (lb/Mcal)
Angus	22,435	1,078	.049
Braunvieh	22,624	1,243	.057
Charolais	17,117	1,243	.055
Gelbvieh	22,036	1,170	.055
Hereford	20,890	985	.048
Limousin	21,786	1,199	.056
Red Poll	20,119	1,130	.058
Pinzgauer	20,186	1,102	.056
Simmental	20,975	1,047	.050

Characterization of Lactation Curves for Nine Breeds of Cattle Fed Differing Rations

Thomas G. Jenkins and Calvin L. Ferrell[1]

Introduction

Genetic merit for milk production influences the weight of calf marketed by producers. Higher preweaning weight gains are made by calves from cows that produce high levels of milk. Lactational productivity can influence future levels of herd calf output if the expression of higher genetic potentials for milk production exceeds the nutrient availability for the production environment. For example, if the lactating female energy requirements exceed the available energy resources, then the ability to reinstall the estrous cycle may be delayed. For producers, this delay may result in younger, lighter calves in the following production cycle. Producers using restricted breeding seasons may find that the number of cows conceiving is reduced. If the producer's management strategy includes culling of once open females, more heifers are required to be retained for replacements, thus reducing the number of young animals for sale.

Previous research has documented that differences exist among breed crosses or breeds of cattle for characteristics associated with lactation. Yield at time of peak lactation and total milk yield during the lactation period vary. Among dairy animals, research has shown that the higher producing animals tend to be in negative energy balance during the first part of the lactation cycle, i.e., in an attempt to achieve their genetic potentialor milk production, the cows produce more energy in milk than they can consume. Feeding strategies have been or are being developed to circumvent this problem. It is not argued here that genetic potential for milk production of beef breeds is directly comparable to dairy cattle, rather that the range in feed energy environments in which lactating beef cows produce offers a similar opportunity for a negative energy balance to occur. Current recommended feeding standards make recommendations for supplemental feeding based on level of production but ignore the possibility of breed differences.

The object of this study was to quantify breed differences for component traits describing the lactation curve among beef breeds and to characterize the response of these traits to increasing feed energy availability.

Procedure

As part of a comprehensive project to evaluate life cycle production efficiency, lactation records of mature cows representing nine cattle breeds were collected from 1987 through 1990. Breeds included were Angus, Braunvieh, Charolais, Gelbvieh, Hereford, Limousin, Red Poll, Pinzgauer and Simmental. Sixteen cows of each breed were assigned to the study. All cows had calved a minimum of two times prior to entrance into the study. At the initiation of the study, cow ages ranged from 5 to 8 yr. Cows were housed in open-front barns with concrete flooring. Each year pregnant cows were transferred to grass pastures for calving. Time on pasture ranged from 14 to 90 days. Ten to 16 days postcalving, cow-calf pairs were returned to the intensive facilities.

Cows received a ground alfalfa hay based diet. Composition of the diet is detailed in Table 1. Within each breed, four cows were assigned to one of four energy intake levels during the lactation period: 170, 210, 250 and 290 kcal ME/wt/-.75/day. Each cow's ration was determined by using the weight of the cow at the 6-7 mo of gestation of the year the cow entered the study. The ration was fed daily, with feed consumption summed and recorded weekly for each cow. Samples of feed were taken daily and composited weekly. These composite samples provided material for determination of dry matter and crude protein.

Milk yields were determined approximately five to seven times from 14 to 196 days postpartum by weigh-suckle-weigh techniques. Separation of cows and calves preceded the sampling time by 17 hr. The difference between calf weights prior to and after suckling adjusted to a 24 hr basis provided an estimate of daily milk production of the cow. Suckling continued for approximately 45-60 min following introduction of the calves to their dams. Cow lactation records with fewer than five daily samplings within a production cycle were excluded from the data set. A total of 431 lactations from 179 cows was included in the data set.

To evaluate lactation curve characteristics, individual animal observations were used to develop lactation curves for each cow. From these curves, three traits were determined:

time of peak lactation
yield at time of peak lactation=
210-day total yield

Time of peak lactation (PK), yield at time of peak lactation (PKYD), and total yield for a 30-week lactation period (TOTAL) were analyzed to determine if differences exist among breeds, level of energy intakes, and the interaction between breed and energy intake. One of the objectives of this study was to determine if the response within a breed to increased metabolizable energy (ME) availability during the lactation period for milk production characteristics differed.

Results

Differences were observed among the nine breeds for PK, PKYD, and TOTAL. Increasing energy intake level increased PKYD and TOTAL but the increase in these traits decreased per unit increased energy intake. The largest increases would be realized at the lower energy intake levels.

Least squares means by breed for all traits are reported in Table 2. Estimated PK (wk) for the Hereford breed occurred earlier than for Angus, Braunvieh and Red Poll, but at a similar time postparturition as the remaining breeds. The Red Poll was similar to the Angus, Braunvieh and Gelbvieh, but differed from the remainder of breeds. The remaining breeds were intermediate and did not differ from one another for PK.

Yield at time of peak lactation was similar for Braunvieh, Gelbvieh, Pinzgauer, and Simmental. These four breeds produced more milk at PK than the British breeds (Angus and Hereford) or Limousin, and Charolais. Total yield of the breeds ranged from approximately 2600 to 4000 lb pooled over energy intake level. Braunvieh yield for a 210 day lactation period exceeded all breeds except for Gelbvieh. The Hereford and Limousin production were similar. Intake level of ME affected all the response variables (Table 3). PK was later for cows fed at 210 kcal ME/wt.$^{.75}$/d than for cows receiving 170 kcal ME (8.3 ± .3 and 9.2 ± .3; respectively). Cows fed at the higher energy intakes differed from these levels but not from each other. Positive response to PKYD

[1]Jenkins is a research animal scientist, Production Systems Research Unit; and Ferrell is the research leader, Nutrition Research Unit, MARC.

was observed with increasing levels of energy intake. A similar positive response occurred for TOTAL. TOTAL yields for 290 and 250 kcal ME energy intake levels were greater than 210, which was greater than 170 kcal ME. The difference between 290 and 250 kcal ME/wt.$^{.75}$ was not significant. Pooled across breeds, curvilinear responses in PK, PKYD and TOTAL were observed as ME allowance increased but the rate of increase decreased as the energy allowance was raised.

Information describing the effect of increasing energy allowances upon lactation curve traits in beef cattle is limited. In comparison with previous information provided for dairy cattle, it is evident that beef cattle respond similarly to increasing levels of energy intake. With increased energy allowance, PK was delayed and the yield at that time increased. The rate or degree to which these changes occur depends on previous energy intake. Breeds of cattle differ in lactational characteristics. Information such as this can be used by producers to identify those breeds that fulfill the needs for a specific production enterprise and perhaps partially define the role for a specific breed in the industry.

Table 1— Composition of diets (% of dry matter)

Ground alfalfa	77.5	
Corn	17.5	
Corn silage	5.0	
Metabolizable energy	1.03	Mcal/lb
Crude protein	16%	

Table 2—Means for time of peak yield, yield at time of peak yield, and total yield for nine breeds of cattle

	Traits		
		Yield	
Breed	Time of peak lactation (wk)	At peak lactation (lb/d)	210 d total (lb)
Angus	10.4	20.7	3,130
Braunvieh	10.3	26.2	3,967
Charolais	9.5	21.6	3,152
Gelbvieh	10.0	25.3	3,733
Hereford	8.8	18.7	2,620
Limousin	8.8	20.9	2,968
Red Poll	11.1	22.2	3,445
Pinzgauer	9.6	24.4	3,608
Simmental	9.6	24.0	3,528

Table 3—Means for time of peak lactation, time of peak yield, yield at time of peak yield, and total yield by energy availability levels of metabolizable energy

	Traits		
		Yield	
Energy intake levels kcal/wt $^{.75}$	Time of peak lactation (wk)	At peak lactation (lb/d)	210 d total (lb)
170	8.3	20.4	2,726
210	9.2	22.7	3,271
250	10.7	23.8	3,661
290	10.9	24.2	3,742

Estimates of Mature Weights and Maturing Rates for Breed Crosses

Thomas G. Jenkins, Miroslav Kaps, Larry V. Cundiff, and Calvin. L. Ferrell[1]

Introduction

Recent attempts to increase weight of product marketed for a cow herd have emphasized increasing the weights of progeny that are to be sold. Previous investigations have identified sufficient variation between and within breeds of cattle to enable the producer to set the desired level of genetic potential for size in the cow herd and rate of growth in the progeny. The assumption has been that a positive relationship exists between mature size and productivity. Researchers are beginning to question if this assumption is correct. It has been reported that mature size is negatively related to productivity. However, higher productivity has been related to faster maturing rate (the rate at which an animal attains its mature body mass). Breeds of cattle would be expected to vary with regard to the combination of maturing rate or mature weight that would be most beneficial for production. With the diversity in genetic potential for weights available, today's producers should be able to set the optimum mature weights and maturing rates for their production goals within a defined production environment. Exploitation of genetic differences among the breeds for these traits has been suggested as a way that beef production efficiency could be improved. Exploitation of these differences requires characterization of measures of growth through maturity for a large number of breeds or other uniquely defined populations of cattle. Estimates of growth parameters from birth through 15-18 months are readily obtainable. Estimates of mature weights and rates of maturing for diverse populations are limited. The objective of the present investigation was to estimate means for breeds for several measures of growth through maturity, thus providing an information base characterizing diverse breeds of cattle with regard to mature weights, rates of maturing, and heights.

Procedure

Data that were analyzed for this report were collected as part of the Germ Plasm Evaluation Program conducted at MARC, a comprehensive investigation conducted to characterize production performance for diverse breeds of cattle. The data for weight, height and condition scores of F_1 females from the first three cycles were produced from 1970 through 1976. The F_1 females used in the study were produced by artificially inseminating either Angus or Hereford cows with semen from Angus, Brahman, Brown Swiss (European and American), Charolais, Chianina, Gelbvieh, Hereford, Jersey, Limousin, Maine Anjou, Pinzgauer, Red Poll, Sahiwal, Simmental, South Devon and Tarentaise bulls. More detailed information describing how sires from the different breeds were identified for use has been previously reported. Weights were recorded at birth, weaning, at 28-day intervals from weaning to approximately 24 mo of age and then twice yearly until a cow was removed from the project. Postweaning, heights at the hip, and body condition scores (9 point; 1 = extremely emaciated, 9 = extremely obese) were recorded at each weighing. Calves born into the project were raised on pasture with their dams and weaned at approximately 200 days of age. Following weaning, the heifers were maintained in a drylot for approximately 168 days. Multiparous cows were sustained on cool- and warm-season grass pastures during the summer months. During the winter, legume and grass hay was provided to the cows. Weight, height and condition measurements were recorded prior to initiation of the breeding season and at time of weaning. Records from individuals that were open two consecutive years were removed from the evaluation. Animals dying prior to accumulating a minimum of 24 measurements of weight were deleted from the data set. Animals were not removed based upon a growth criterion. These edits resulted in a set of records collected from 1577 individuals that were used for analyses. The number of sires for each of the breeds were: Angus 32; Brahman 17; Brown Swiss 11; Charolais 25; Chianina 17; Gelbvieh 11; Hereford 32; Jersey 32; Limousin 12; Maine Anjou 15; Pinzgauer 9; Red Poll 16; Sahiwal 6; Simmental 26; South Devon 29 and Tarentaise 6; respectively. For greater detail concerning management protocol of cattle assigned to the Germ Plasm Evaluation Program, see previous progress reports.

Breeds included in the project were identified for evaluation to characterize the diversity in potential for production traits such as growth rate, age at puberty, and milk producing ability. To minimize the effect of variation in body condition on weights of animals within each breed attributable to individual animal differences in milk production, weight measurements recorded after 20 mo of age were adjusted to a constant condition score. Growth curves (weight-age relationship) were estimated for the individual animals using the recorded weights. Nonlinear regression was used to fit animal weights to a growth function commonly referred to as the Brody growth model. An assumption associated with this model is that the rate of growth along the growth curve is proportional to the amount of growth remaining to be attained. Parameters of interest estimated from this model include the asymptote of the curve (A) and the rate constant (k), respectively, which previously have been used to describe mature weight and rate of approach to mature weight of living organisms.

Results

Improvement in production characteristics measured by weight may result from selection within breeds or by using breed substitution to exploit direct genetic variation among breeds. Based upon previous research, immediate improvement could be realized by breed substitution. However, within breed selection represents the beneficial course of action to maintain genetic diversity among our cattle breed populations. Breed means and characterizing traits of interest are reported in Table 1. Information such as this can be used to identify the breed resources for the most appropriate breeding program. In addition to mature weights, maturing rate and height at maturity, weights at birth, 200 day, 365 day, and 500 day are reported for the 16 sire breeds. Weights for the traits averaged over breeds of sire were 77, 427, 645, 741, 1140 lb for birthweight, weaning weight, yearling weight and 500 day weight. The average weight of maturing was 5.6% and the average height at maturity was 50 in. Cows sired by Chianina bulls and those sired by Maine Anjou bulls exhibited heavier mature weights and attained these weights at relatively slow rates (maturing

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rates approximately 16% less than the average of the 16 breeds). The mature weight average of the Jersey sired cows was approximately 18% less than the pooled breed average and, as indicated by their maturing rate, they attained their mature weight rapidly. The Brahman, Hereford, Sahiwal and Tarentaise sired females grew to a heavier mature weight than the Jersey sired female but the maturing rate averages for these breeds were similar to that of the Jersey. Using information from this study in combination with results from other studies, producers should be able to identify breeds of animals which are most suited to serve a paternal, maternal, or general purpose role in the beef industry today. The ability to exploit differences among breeds to establish desired mature sizes or rates of maturing is evident from the diversity among the sire breeds reported in this study. Using mating systems to effectively use this genetic diversity should provide the producer an effective means to improve the efficiency of beef production.

Table 1— Mature weight, maturing rate, height at maturity, and weights at various ages by sire breed

Sire breed	N	Weight (lb)[a]					Maturing rate[a] (k*100)	Height at maturity(in)
		Birth	200 d	365 d	500 d	Maturity		
Angus	122	75	394	613	715	1126	96.4	49.2
Brown Swiss	107	84	427	638	743	1144	98.2	50.4
Brahman	94	81	462	715	823	1210	108.9	52.0
Charolais	100	81	451	651	750	1219	89.0	50.8
Chianina	67	87	429	653	770	1296	83.9	53.5
Gelbvieh	65	84	440	649	761	1185	94.6	50.4
Hereford	173	73	431	653	737	1100	107.1	48.4
Jersey	99	62	376	552	634	935	108.9	48.4
Limousin	136	75	422	618	704	1135	89.3	50.4
Maine Anjou	67	90	429	667	779	1283	87.5	51.2
Pinzgauer	85	92	451	719	805	1155	117.8	50.4
Red Poll	81	81	403	592	695	1124	87.5	49.2
Sahiwal	83	75	431	658	748	1069	110.7	50.0
Simmental	132	81	444	661	752	1131	101.2	50.8
South Devon	93	77	416	627	719	1126	96.4	52.0
Tarentaise	73	75	442	701	783	1142	112.5	49.6
Pooled	1577	77	427	645	741	1409	5.6	50.0

[a] Maturing rate of each sire breed is expressed relative to the pooled estimate of 5.6.

Simulated Effects of Herd-Level Management Strategies on Efficiency of Beef Production

Michael D. MacNeil, Don D. Kress, and Gordon E. Dickerson[1]

Introduction

Beef producers make some decisions that affect production at the herd level. In many cases these decisions are not supported by data, since resources needed to obtain experimental data are limited. Computer modeling is a logical way to evaluate herd level effects. Here we examine options for culling cows based on age and pregnancy status under four crossbreeding systems.

Procedure

We used a computer model to calculate inputs and outputs needed for beef production. This model followed from earlier work at Texas A&M University and at MARC. Monthly estimates of diet digestibility for each class of stock described the production environment. Classes of cattle included cows, replacement heifers, and steers and surplus heifers destined for slaughter. We summed all inputs and outputs needed to produce, on avg, low choice carcasses. Conclusions from these results apply to herds where management retains ownership until steers and surplus heifers are marketed at a grade constant (low choice) endpoint.

Input costs for cows and replacement heifers were obtained from a survey of production costs per cow unit in the ranching area of Nebraska. Costs for inputs included: grazing at $13/animal unit mo, native hay at $35/ton, protein supplement at $190/ton, other cash costs (including interest) $98/cow, and labor at $6/hour. Input costs for feeding calves were from a similar survey associated with feeding steers from weaning to market weight on corn-based high concentrate rations. These costs included: corn at $2.25/bushel and other cash costs including interest at $79/head. We used ten-yr (1977-1986) avg prices paid for beef cattle in Nebraska to calculate returns. These avg prices per lb were: $0.61 for choice market-ready steers, $0.59 for choice market-ready heifers, and $0.39 for cull cows.

We examined four mating systems to study gains obtained from either separately or jointly using heterosis and breed differences. A straightbred system makes the most use of breed differences, using only the single "best" breed, but does not capitalize on heterosis. A three-breed rotation captures 87% of available heterosis, but takes less advantage of breed differences than the straightbred system. Terminal sire systems use both heterosis and breed differences to varying degrees. We simulated a roto-terminal system and a specific cross system. The roto-terminal system is a maternal two-breed rotation with terminal sires bred to cows 4 yr old and older. First-cross females are produced and then bred to terminal sires in the specific cross system. Except for the straightbred system, all systems require three breeding pastures. Cows simulated had 1,100 lb genetic potential for mature weight and 33.7 lb/day maximum milk yield. Genetic potential of terminal sires for mature size was 40% greater than for the females.

Cows and yearling heifers were bred during June and July. Culling options varied in severity of culling open females. They were: 1) no culling based on pregnancy status, 2) open heifers culled, 3) open heifers and open 2-yr-olds culled, 4) open cows, 2-yr old and older culled, and 5) all open females culled. We also simulated culling of cows as they reached maximum ages of 7, 9, 11, 13, or 15 years. All imposed culling took place at weaning, on November 1. Model calculations set equilibrium age distributions so that 1,000 simulated females always entered the breeding season.

Biological efficiency was defined as total TDN input to the production system divided by total slaughter wt equivalent output. Slaughter wt equivalent output was total slaughter wt of steers and surplus heifers plus .63 times the slaughter wt of cull cows. Thus, slaughter wt of cull cows is valued at 63% of slaughter wt of steers and heifers. Economic efficiency was total cost attributed to the production system divided by total value of its outputs. Net return was the difference between total value of outputs from the production system and total cost of its inputs.

Results

Relative importance of mating system, culling option, and maximum cow age depended on whether the evaluation was at a biological or economic endpoint. The five options for culling based on pregnancy status were the largest contributor (40%) to differences in biological efficiency. Mating systems (27%) and culling at different maximum ages (28%) were also important contributors to biological efficiency. However, mating systems had the greatest effects on economic efficiency and net return, 56% and 60%, respectively. Culling options based on pregnancy accounted for only 21% of variation in economic efficiency or net return. Culling at a prescibed maximum age contributed only 20% and 16% to differences in economic efficiency and net return. Within this simulated production environment and with avg production costs and prices received, optimal management resulted in $1.02 of expense for every $1.00 of income.

Interactions of culling strategies and mating systems were not important to understanding differences in either measure of efficiency or in net return. Therefore, the consequences of culling strategies and mating systems can be discussed separately. Likewise, interpretations of differences among mating systems, among culling strategies based on pregnancy status, and among maximum ages were consistent across both efficiency measures and net return. Therefore, graphical presentations were limited to economic efficiency or cost per $1 of income.

Among the mating systems studied, the straightbred system was least efficient (Fig. 1). It required 10.05 lb of TDN for every lb of slaughter wt equivalent output. Economically, inputs costing $1.68 returned $1.00 in income and the net return per cow was -$105 for the straightbred system. By using heterosis, but not breed differences, the three-breed rotation system improved biological efficiency to 9.65. For the three-breed rotation, economic efficiency was 1.60 and net return was -$86. Using a terminal sire with a two breed maternal rotation was most efficient. In that system with bio-

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logical efficiency at 9.16, economic efficiency was 1.43 and net return was -$29. The three-breed specific cross system was intermediate between the three-breed rotation and using terminal sires in combination with a two-breed rotation.

In Figure 2, we present simulated effects of culling based on pregnancy status. Culling open females at all ages improved herd-level efficiency and profitability. When keeping open females, 9.96 lb TDN produced a lb of slaughter wt equivalent output. Economically, inputs costing $1.61 returned $1.00 and net return per cow was -$82 when not culling open females. Culling all open females improved biological efficiency to 9.07. Economic efficiency was 1.48 and net return per cow was -$48 when culling all nonpregnant females.

Shown in Figure 3 are effects of maximum cow age on economic efficiency. Results for biological efficiency and net return were similar. We did not find an optimal maximum age at which to cull cows in this study. Keeping cows as long as they remain sound was the most efficient and profitable strategy simulated. However, the decreasing rate of improvement in efficiency probably results from the relatively small number of cows remaining at the older ages. These results may be sensitive to assumptions about involuntary culling at older ages.

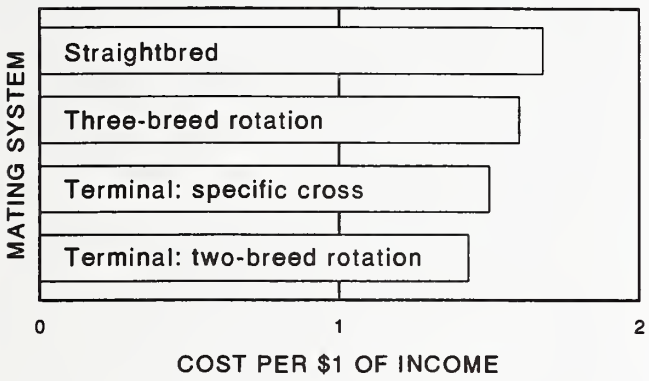

Figure 1 – Mating system effects on economic efficiency.

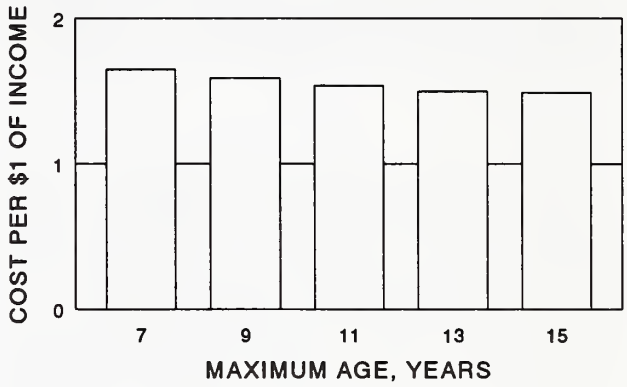

Figure 3 – Effects of maximum cow age on economic efficiency.

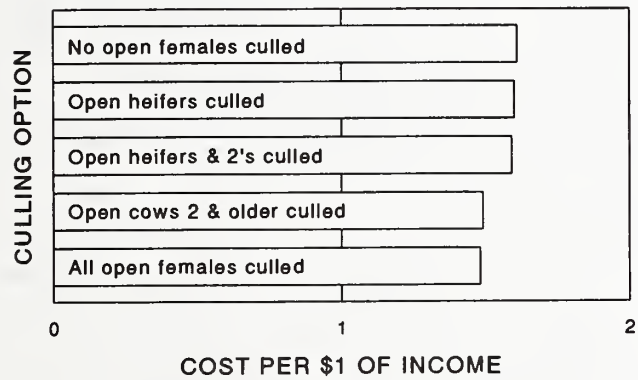

Figure 2 – Effects of culling open females on economic efficiency.

Using Crossbreeding Systems to Produce Beef

Michael D. MacNeil, Larry V. Cundiff, Keith E. Gregory, and Robert M. Koch[1]

Introduction

Crossbreeding provides an opportunity to improve performance by beef cattle. Breed differences are heritable and can be used to produce superior crossbred cattle. Heterosis results from bringing together unlike genes from different breeds to produce an animal with a level of performance that exceeds the average of the parent breeds. We develop crossbreeding systems to make the greatest improvement in performance possible consistent with a sustainable breeding program. Heterosis and differences among breeds are tools of the trade. In this paper, we combine the results from earlier studies to investigate their practical applications.

Procedure

Angus, Hereford and Shorthorn cows produced straightbred and two-breed cross calves. Resulting straightbred heifers were bred to bulls of the other breeds to produce additional two-breed cross calves. Two-breed cross heifers resulting from the original matings produced three-breed cross and backcross progeny at the same time. The three-breed cross and backcross heifers formed the foundation for two- and three-breed rotation systems. These rotation systems continued for two more generations. Straightbred calves were produced along with backcross, three-breed cross, and rotation calves. All calves grazed with their dams until weaning and had no access to creep feed.

Steer calves were fed a growing-finishing ration containing 1.18 Mcal metabolizable energy per lb for 252 days after weaning. Then they were slaughtered and carcass data collected.

Breed group differences resulted from breed effects of the individual, its dam, or its maternal grandam. Heterosis at each generation also may contribute to differences among breed groups. Data analyses quantified the effects of substituting one breed for another and the effects of heterosis on weaning wt, final wt, carcass wt, and retail product wt per cow exposed.

Terminal sires express genetic effects only through direct influences on their offspring. For this study, we characterized a generic terminal sire breed using results from the Germ Plasm Evaluation Program. Traits of a terminal sire affecting wt produced per cow exposed include: calf mortality to weaning, calving date, birth wt, preweaning daily gain, postweaning daily gain, carcass wt, marbling score, and retail product weight. The basis for the terminal sire breed was expression of these traits by calves from Brown Swiss, Gelbvieh, Maine Anjou, Simmental, Limousin, Charolais, and Chianina sires compared with calves from Hereford and Angus sires.

Thus, Angus, Hereford, Shorthorn, and terminal sire breed resources were available. Using these resources, we then predicted performance of five mating systems. The systems considered were: straightbred, two-breed start rotation (Fig. 1), three-breed rotation, and two- and three-breed maternal rotations with a terminal sire (Fig. 2).

Results

The mating system of choice depends on several resources that are specific to each cattle operation. These resources include: number of cows, number of breeding pastures, availability of labor, and amount and quality of feed and forage. We assume breeding of all cows was by bulls in natural service. Compromising a system by failing to meet its requirements reduces the benefits that can be expected from it. Resources required to put the mating systems into place are reviewed here.

The straightbred system is the simplest to carry out. Its success requires appropriate matching of available feed resources and environment with an adapted breed. Numbers of cows and breeding pastures, managerial ability, and availability of labor are least restrictive to the straightbred system of all mating systems.

A two-breed rotation requires enough cows to employ two bulls, two breeding pastures, and identifying all females by the breed of their sire. A three-breed rotation requires a correspondingly greater commitment of resources. In either rotation, bulls are bred to cows that are most distantly related to the breed of the bull. Rotation systems provide limited opportunity to take advantage of breed differences. Environmentally well adapted breeds that are comparable in birth weight, growth, and lactation potentials should be used.

Terminal sire based systems allow use of breeds in specialized roles. Young cows bred in a rotation among breeds that are superior for maternal traits and adapted to the environment produce the replacement heifers. Mature cows are bred to breeds with high genetic potentials for growth rate and lean-to-fat ratio of the carcass. All progeny of the terminal sire are sold for slaughter. Using a terminal sire with a two-breed maternal rotation requires three breeding pastures and enough cows to use four bulls.

All crossbreeding systems produced more lb of product per cow exposed than the straightbred system. Products considered were weaning wt (Fig. 3), final wt (Fig. 4), carcass wt (Fig. 5), and wt of retail cuts (Fig. 6)

Heterosis increased weaning wt per cow exposed from the two-breed rotation by 59 lb and from the three-breed rotation by 75 lb over the straightbred system. Adding a terminal sire to a rotation system yielded only small increases in weaning wt per cow exposed. Three-breed rotation and terminal sire on two-breed maternal rotation systems require similar numbers of breeds. Yet the latter system produced only 3.7 lb more weaning wt per cow exposed. We conclude cow-calf producers should evaluate a terminal sire system carefully before deciding to use it.

Comparing among endpoints preceding slaughter, the various crossbreeding systems were similar to the weaning endpoint. Only when we examined retail product wt per cow exposed did important advantages (26 lb or 18%) lie with the terminal sire systems over the rotation systems. If consumers want leaner meat products, then economic benefits from producing calves using terminal sires need to be transferred from consumers back to cow-calf producers.

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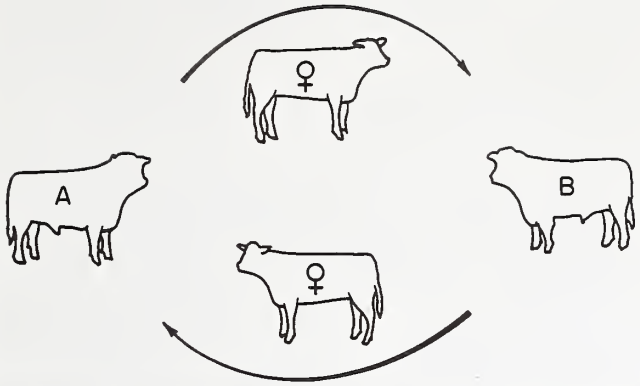

Figure 1 – Two-breed rotation. Bulls of breed A are bred to females sired by bulls of breed B. Bulls of breed B are bred to females sired by bulls of breed A.

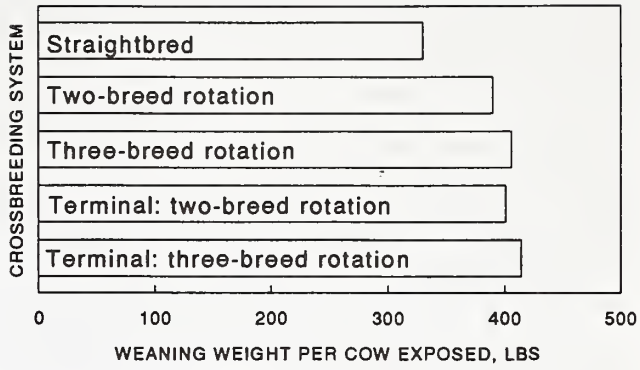

Figure 3 – Effects of crossbreeding system on weaning weight per cow exposed.

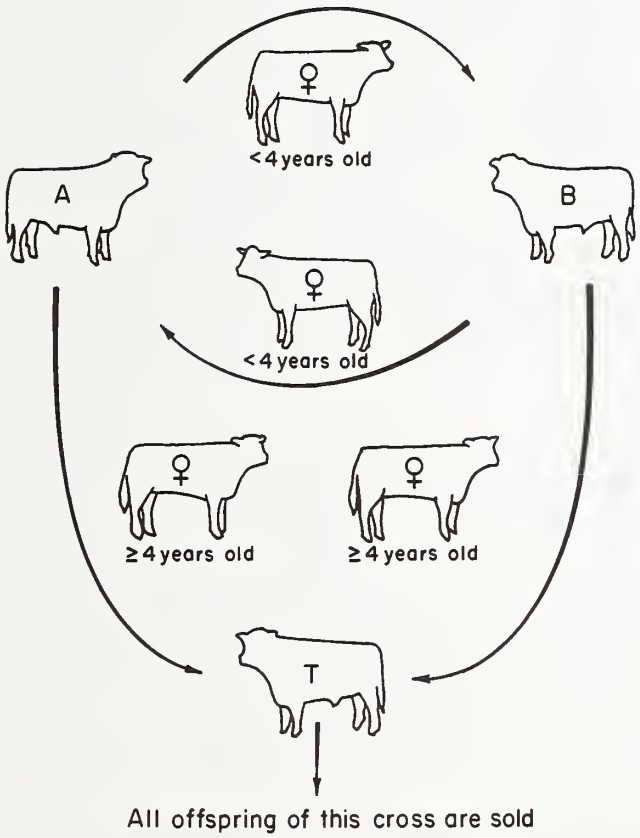

Figure 2 – Terminal sire incorporated with two-breed rotation. Heifers and cows less than four years old are bred in a two-breed rotation. Older cows are bred to bulls of the terminal sire breed and all offspring from the terminal sire breed are marketed.

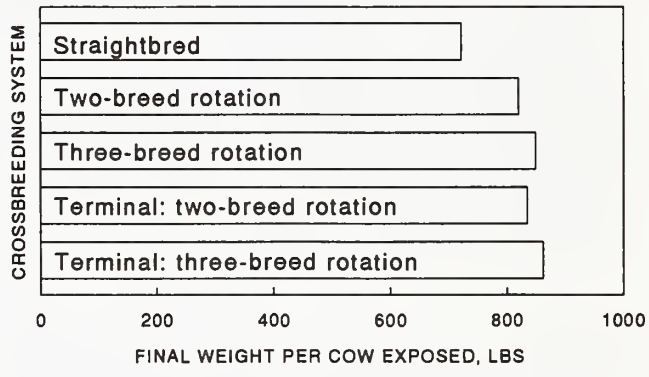

Figure 4 – Effects of crossbreeding system on final weight per cow exposed.

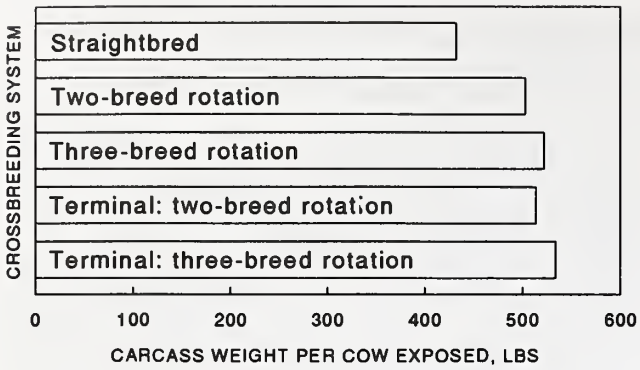

Figure 5 – Effects of crossbreeding system on carcass weight per cow exposed.

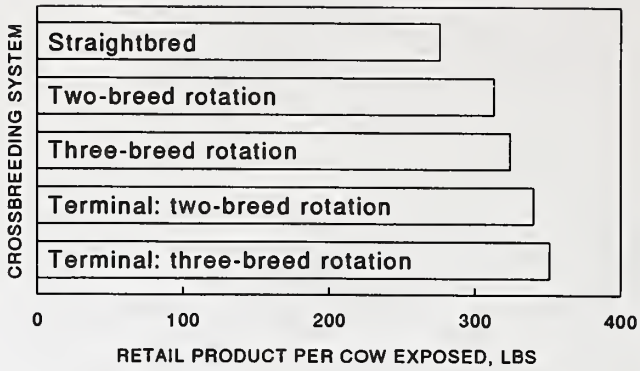

Figure 6 – Effects of crossbreeding system on retail product weight per cow exposed.

Effects of Inbreeding and Heterosis in Hereford Lines on Reproduction and Maternal Performance

Michael D. MacNeil, Delwyn D. Dearborn, Larry V. Cundiff, Chris A. Dinkel, and Keith E. Gregory[1]

Introduction

Two genetic mechanisms have been described as potential explanations for heterosis. The first mechanism is dominance. Dominance occurs when there are two differing forms of a gene (alleles) at a given position (locus) on a pair of chromosomes and where one of the pair of alleles masks or over powers the effect of the second. Having two different alleles at a locus is referred to as heterozygosity and the affected individual is heterozygous. Whether an individual has one or two copies of a dominant allele makes little difference in its superiority over others having two copies of the recessive allele. A higher degree of heterozygosity is expected when one population carrying the dominant allele in high frequency is crossed with a second population carrying the recessive allele in high frequency. Alternatively, heterosis may result from joint effects of genes at several loci. This alternative mechanism is called epistasis.

Previous research documents reduced performance resulting from the mating of closely related individuals (inbreeding). Inbreeding generally reduces growth and reproductive rates and delays maturity. This inbreeding depression arises from increasing the frequency with which two alleles at a locus are identical (homozygous) and again coupled with dominant gene action. Thus, effects of inbreeding and heterosis are of similar size but opposite in direction, if dominance at individual loci causes both.

In this study, we used inbreeding and linecrossing of Hereford cattle in an attempt to distinguish between these two explanations for heterosis influencing maternal traits. Answering this question sheds light on the amount of heterosis to be expected in composite breeding schemes.

Procedure

Scientists with the South Dakota Agricultural Experiment Station created four inbred lines. Each inbred line started from 1 bull and 15 cows. The same 4 bulls and 60 cows were the basis for a contemporary control line. The control line was maintained as a single herd. Mating bulls from each inbred line with cows from the other inbred lines resulted in production of linecross females. Mating inbred bulls to control line cows produced topcross females. Mating of related cows and bulls was avoided in producing topcross females. Replacement females (control, inbred, linecross, and topcross) were transported to MARC and evaluated for reproductive and maternal performance over an eight-yr period.

Results

Performance of females from the four lines as 2-yr-old heifers and at all ages is shown in Table 1. The topcross breed group can be used to separate effects of inbreeding of sire and dam. In this study, the topcross breed group did not differ in performance from either the linecross or control breed groups.

If performance of inbred and control lines differs, then effects due to inbreeding exist. Inbreeding depressed survival of calves from pregnancy testing to calving of first calf heifers. Birth weights of calves from inbred cows were also lighter than from control line cows. Except for pregnancy rate, other comparisons of inbred and control line cows were also negative. However, they were not large enough to establish conclusively the existence of inbreeding effects.

Heterosis exists if performance by linecrosses differs from that of the parental inbred lines. Survival rates for calves from linecross females exceeded those from inbred females both from pregnancy testing to calving and from calving to weaning. Linecross cows also had heavier calves than inbred cows, both at birth and at weaning.

Comparing effects of heterosis and inbreeding, we find no differences in their size for any trait except birth weight. For birth weight, inbreeding depression was larger than heterosis. This result may stem from the heavier than expected birth weights of calves from control line cows. Results of this study indicate that effects of inbreeding are detrimental to reproduction and maternal performance in cattle. Crossing inbred lines results in significant heterosis. Performance levels of linecrosses apparently are restored to the level of noninbred contemporaries.

Table 1—Levels of inbreeding, reproductive traits of two-year-old heifers and maternal performance of inbred, linecross, topcross, and control line cows

	Breed group			
Traits	Inbred	Linecross	Topcross	Control
Level of inbreeding, percent				
Individual	27	0	0	7
Sire	31	34	27	4
Dam	24	27	7	6
2-yr-old				
Pregnant, percent	76	79	70	76
Prenatal survival, percent	85	97	97	100
Birth rate, percent	66	78	68	77
Postnatal survival, percent	70	90	80	83
Weaning rate, percent	46	70	55	65
All ages				
Birth wt, lb	72	75	76	82
Weaning wt, lb	400	429	432	431

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Postpartum Interval Is Influenced by Nutritional Environment and Biological Type

Russell A. Nugent III, Thomas G. Jenkins, Andrew J. Roberts, and John M. Klindt[1]

Introduction

Reproduction is a major component of production efficiency for a cow-calf system. Failure of a cow to conceive is the most important factor reducing net calf crop. The interval from parturition to estrus, or postpartum interval, greatly influences the chances of a cow becoming pregnant during a restricted breeding season. Breeds can differ in length of postpartum interval and further, postpartum interval is influenced by numerous environmental factors including nutritional value of available feedstuffs. Inadequate energy availability increases postpartum interval in suckled beef cows, but energy requirements differ among biological types. The level at which energy begins to limit reproductive performance may not be constant for all biological types of cattle. The objective of this study was to test the effects of biological type and daily metabolizable energy availability on length of postpartum interval of mature beef cows.

Procedure

Mature, multiparous purebred Angus, Braunvieh, Charolais, Gelbvieh, Hereford, Limousin, Pinzgauer, Red Poll, and Simmental cows were randomly assigned within breed (four cows per level, 144 total females) to be fed for 4 yr at either 130, 170, 210, or 250 kcal of metabolizable energy x body weight$^{-.75}$ per day during nonlactational periods, with an increase in ration size of 25% during lactation. The diet is outlined in Table 1. Body condition scores (1 = very thin to 9 = very fat) averaged over breeds were approximately 1.5 to 3.5 for cows fed at 130 kcal and 7.0 to 8.5 for cows fed at 250 kcal. The other two energy levels yielded intermediate condition scores.

Prior to treatment, cows were grazed on mature smooth brome pasture, and the body weight of a cow at the time she entered the study (avg day of gestation: 210; range: 189-231) was used to establish her individual ration throughout the remaining time on the study. Average age of cows was 9 yr (range: 5-13), and avg age did not differ between nutritional treatment X biological type subclasses.

Prior to the study, breeds were assigned to biological types based on genetic potential for mature size (growth) and daily yield of milk at time of peak lactation (milk). Genetic potentials for growth and milk were determined from previous studies at MARC. The four biological types were: moderate genetic potential for milk and growth (Angus, Hereford, Red Poll), moderate genetic potential for milk and high genetic potential for growth (Charolais, Limousin), high potential for milk and moderate potential for growth (Braunvieh, Pinzgauer), and high genetic potential for milk and growth (Gelbvieh, Simmental).

Cows of the same breed and treatment were housed together in 760 square ft open front barns. Individual feeding was accomplished through use of electronic headgates. Two wk before the expected earliest calving date, all pregnant cows were transferred to pasture for calving. Cow-calf pairs were returned to feeding pens at approximately 2 wk after calving. Calves were weaned at approximately 200 days of age (range was 175 to 225 days). Prior to statistical analysis, effects of calf date of birth were removed from the data.

In 1991, the 121 (out of 144) cows that calved were bled once per wk starting 3 wk postpartum. Blood samples were collected for 27 wk postpartum on 31 cows and for 15 wk postpartum on 90 cows. Cows were not exposed to bulls following calving in 1991. Circulating concentration of the reproductive hormone progesterone was then determined from each blood sample by radioimmunoassay procedures. Postpartum interval was defined as the number of wk from parturition to the beginning of the first normal length luteal phase (the first wk that baseline progesterone preceded 2 wk of elevated progesterone).

Results

Biological type interacted with nutritional treatment to influence postpartum interval (Table 2). Increased energy availability tended to decrease postpartum interval in all biological types, but the magnitude of the decrease depended upon biological type. The interaction between type and nutritional level was, therefore, not one of reranking but rather one of differences among types in magnitude of the decrease in postpartum interval from the lowest to higher energy availability levels. Averaged over type, the 130 kcal energy availability level yielded the longest interval from calving to resumption of cyclicity and 210 and 250 kcal the shortest interval.

Biological types with a high genetic potential for growth exhibited the longest postpartum intervals, but also showed the greatest positive response to increased feed availability. Averaged over treatments, postpartum intervals were longest for the moderate milk, high growth type and were intermediate and shorter for high milk, high growth type cows. Further, increasing energy from 130 kcal to 250 kcal decreased postpartum interval the most for the moderate milk, high growth type. When feed availability was lowest, high genetic potential for peak milk yield decreased postpartum interval by 39 days when associated with high growth potential but increased the interval by 1 day for moderate growth types.

Efficient production dictates that calving intervals and thus postpartum intervals be relatively short. In the present study, the length of the postpartum interval varied between cows that were fed for long periods at specified levels of energy availability. Decreases in postpartum interval in response to increasing energy availability depended upon genetic potential for both mature size and level of peak milk yield. At the lowest level of energy availability, biological types with the greater genetic potential for mature weight exhibited extended postpartum intervals. However, in types with higher genetic potential for milk as well as growth the effect of low energy availability on postpartum interval was greatly reduced.

Thus it appeared that if daily energy availability was limiting, breeds that were historically selected for large mature size and growth (draft and beef) with no accompanied selection for milk (e.g., Charolais, Limousin) may partition a greater portion of their energy intake towards basal metabolism, growth, and lactation before any remaining nutrients are used for resumption of estrous cycles. Conversely, historical selection of a breed for dual-purpose as a milk-beef type (large mature size and high milk output, e.g., Gelbvieh, Simmental) may have resulted in a biological type that partitioned relatively more energy towards reproduction under

[1]Nugent is a research associate and Jenkins is a research animal scientist, Production Systems Research Unit, MARC; Roberts is a research physiologist, Reproduction Research Unit, MARC; Klindt is a research physiologist, Nutrition Research Unit, MARC.

conditions of limited energy availability and therefore returned to estrus earlier.

In cattle populations developed for meat and milk, timely reproductive performance was apparently considered a favorable attribute and presumably selection for milk output was accompanied by direct or indirect selection for other maternal characteristics as well (i.e., age at puberty and postpartum interval). It should be noted that sampling could have influenced results and conclusions should not be interpreted as being necessarily indicative of all cattle from the breeds and biological types used.

Increased genetic merit for level of milk yield had a less marked influence on the effect of energy restriction upon postpartum interval in types with a more moderate weight at maturity. This observation was supported by previous work that showed no difference in postpartum interval among breed crosses with similar moderate mature size, but different genetic potentials for milk production.

Effective production systems require the matching of genetic potential for performance (output) to the environment. Breeds or breed-crosses used to generate and maintain a cow herd must be chosen to some degree for the specific nutritional environment in which they will be producing. Differences among biological types for some component(s) of reproduction appeared to be especially important when energy availability was restricted. Important genotype environment interactions can influence reproductive potential and thus the efficiency of a cow-calf production system.

Table 1 – Composition of diet on a dry matter basis

Ground alfalfa	77.5%
Whole shelled corn	17.5
Corn silage	5.0
Metabolizable energy	1.0 Mcal/lb
Crude protein	16%

Table 2 – Mean postpartum interval by biological type and nutritional environment

Biological type	Daily energy availability[a]				Average by type
	130	170	210	250	
Moderate growth - moderate milk	88	83	46	59	69
High growth - moderate milk	146	69	58	66	85
Moderate growth - high milk	89	91	47	32	65
High growth - high milk	107	70	63	49	72
Average by energy availability	108	78	54	52	73

[a] kcal metabolizable energy x body weight$^{-.75}$ during nonlactational periods: energy availability was increased by 40 kcal during lactation.

Computer Simulation of Body Composition in Growing and Finishing Beef Cattle

Charles B. Williams, John W. Keele, and Gary L. Bennett[1,2]

Introduction

The National Institute of Health Consensus Development Conference in 1985 recommended that Americans eat a diet with no more that 30% of the calories coming from fat, to reduce the risk factors associated with cardiovascular disease. Beef with a low-fat content could compose a greater portion of this recommended diet than beef with a high-fat content. There is a large base of experimental results on the effects of various factors such as genetics, feeding level, sex condition, exogenous biological growth stimulants, time on feed, and postweaning management on growth, composition and palatability of beef carcasses. Systems analysis through the use of computer models is an excellent means of integrating this existing knowledge. Computer models can be used to help identify feeding systems to produce leaner beef, provided these models are general enough to predict body composition with reasonable accuracy. Results from previous research have shown that some differences in body composition of cattle of the same breed and body weight may be predicted by rate of gain. Our objective was to develop and evaluate a dynamic computer simulation model that uses rate of gain to predict differences in body fat caused by plane of nutrition and to identify the model's range of applicability.

Procedures

Previous models that account for differences in body composition among cattle of similar genotype and weight caused by differences in energy intake require nutrient intakes as inputs. Nutrient intakes are difficult to predict and expensive to measure when animals are grazing. Our model is based on the premise that it is easier to predict or measure the growth patterns of cattle on a given nutritional regimen based on past experience or data than it is to predict or measure their nutrient intakes. The model assumes that protein is adequate for the amount of energy consumed. This restriction is based on the assumption that the costs relative to benefits of achieving protein adequacy are small compared to the costs relative to benefits of achieving energy adequacy.

Previous research has shown that daily gains for fat free matter and fat respond to increasing amounts of energy consumption as shown in Figure 1. Assuming the relationships in Figure 1, the percentage of fat free matter in gain decreases with increasing rate of empty body gain in a curvilinear fashion (Figure 2). The relationship shown in Figure 2 was used as a basis for developing a model that uses differences in rate of empty-body gain (caused by differences in energy consumption) to predict differences in body composition. The shape of the curve in Figure 2 depends on genotype, sex, stage of maturity (fraction of mature fat free matter in the body) and previous growth pattern. The model incorporates adjustments for these factors.

The model was evaluated with data from one unpublished and seven published experiments (Table 1). These experiments used several breeds of cattle, growing at rates that varied from small daily losses to high daily gains and various combinations of these growth rates. Ability of the model to simulate animal responses was first evaluated with respect to the accuracy with whichthe model simulated treatment means for fat percentage observed in the experiments. If the model simulated the observed animal responses closely, then paired values (experimental and simulated) should have a relationship which is close to one to one. Second, if there were important differences in body composition of animals at the same body weight, and these differences were associated with differences in nutrition, we wanted to evaluate the ability of the model to account for these nutritional effects.

Results

Observed and simulated treatment means for body fat percentage for the experiments listed in Table 1 are plotted in Figure 3. Data points lie close to the 45 degree line, which supports a one to one relationship between observed and simulated treatment means. However, the data plotted in Figure 3 do not distinguish between differences associated with weight and nutritional effects on body composition beyond those associated with body weight. To address this problem, observed and simulated nutritional effects on percentage body fat were obtained after adjusting for body weight differences. These nutritional effects are plotted in Figure 4. The results show that for experiments in which nutritional treatments had a significant effect on observed composition, the simulated data from the model also showed a similar significant effect. The results depicted in Figure 4 provide evidence that the model can predict differences in fatness of cattle caused by nutrition.

One of the outliers in Figure 4 represents a treatment in which a low protein diet was fed. In this case the model did not predict a significant nutritional effect on composition. One of the underlying assumptions of the model is that dietary protein is adequate, so inadequate protein may be responsible for the conflicting results obtained in simulating this experiment. Large effects of nutrition independent of changes in body weight are probably slightly underpredicted by the model, and the model will haveapproximately the same degree of accuracy in predicting composition as body weight alone in cases for which there are no nutritional effects on composition.

The following is a description of several areas where this model may be appropriate as a research/management tool.

1. Identification of postweaning systems of beef cattle production which would result in leaner carcasses at slaughter.
2. Characterizing the postweaning biological efficiency of different breed types when grown under different postweaning systems of production.
3. Identification of production systems to produce beef for different speciality markets.

In addition to these applications the model can be integrated into larger system models of the entire beef production system.

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[1]Williams and Keele are research animal scientists, and Bennett is the research leader, Production Systems Research Unit, MARC.

[2]The full report of this work was published in J. Anim. Sci. 70:841-866, 1992.

Table 1—Brief description of the experiments used to evaluate the model

Category	n	No. dietary treatments	No. slaughter groups per treatment	No. in initial slaughter group
Holstein steers	47	4	1	8
Angus steers	29	3	4	2
Hereford steers	37	4	2	0
Hereford females	35	4	2	0
Holstein steers-1	54	6	3	4
Holstein steers-2	48	4	3	4
Angus steers	71	2	5	0
Holstein steers	69	2	5	0
Angus steers	42	2	2	12
Charolais steers	41	2	2	12
Small frame	120	6	2,3	0
Large frame	120	6	2,3	0
Small frame	79	5	2,3	10
Large frame	82	5	2,3	10

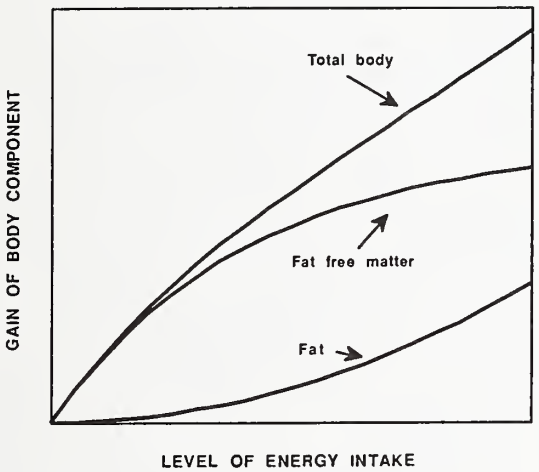

Figure 1 – Relationship between energy intake and daily gain of body chemical components when energy is the most limiting nutrient.

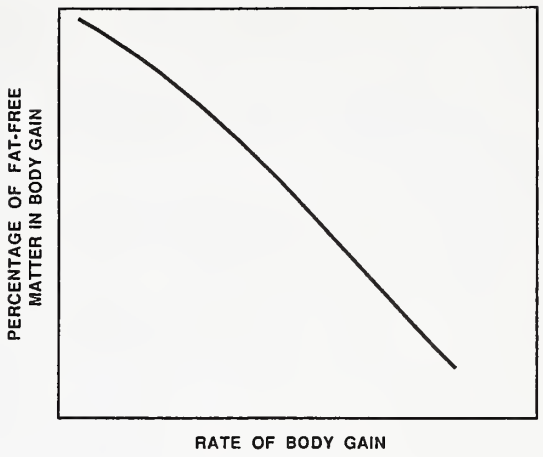

Figure 2 – Relationship between percentage fat free matter in gain and rate of body gain when differences in rate of body gain are caused by differences in energy consumption and energy is the most limiting nutrient.

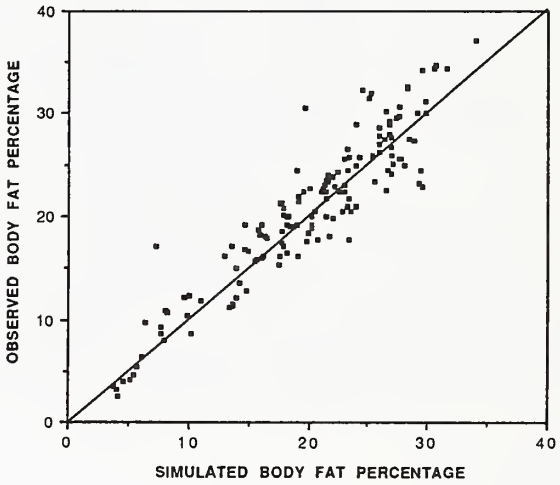

Figure 3 – Relationship between treatment means for observed body fat percentage and simulated body fat percentage.

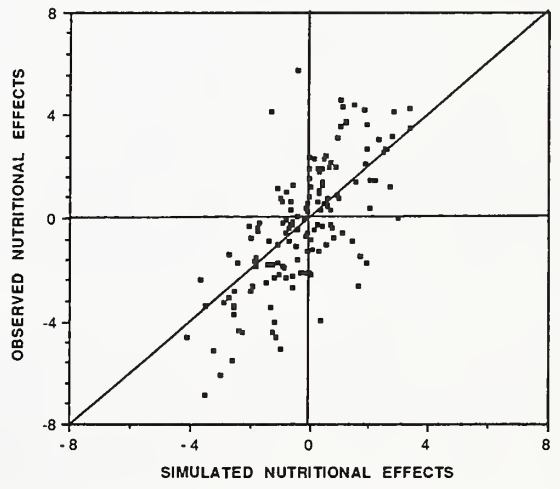

Figure 4 – Relationship between observed and simulated nutritional effects on body fat percentage.

A New Approach to Estimating Empty-Body Weight in Growing and Finishing Beef Cattle

Charles B. Williams, John W. Keele, and Dale R. Waldo[1]

Introduction

Animals require nutrients for maintenance and production. A large part of the calculated nutrient requirements is based on body weight, which includes the contents of the gastrointestinal tract (gut). Ruminants have a large gut capacity, and for a 1000 lb steer, gut contents can account for 50 to 250 lb of its body weight. These contents are not a part of the animal and should not be considered when calculating maintenance requirements. Therefore to translate nutrient requirements for each unit of empty-body weight (body weight minus the weight of gut contents) gain into requirements per unit gain in body weight, we need an accurate method of estimating the weight of gut contents. Several systems have been proposed to estimate empty-body weight. The National Research Council and the Agricultural Research Council used equations to calculate empty-body weight as a constant fraction of shrunk-body weight, or a constant fraction of body weight within three discrete dietary classes, respectively.

Results of previous research have demonstrated that in addition to body weight there is a continuous relationship between weight of gut contents and dietary characteristics such as percentage of dietary concentrates and neutral detergent fiber (indigestible and slowly digested fractions of the feed). Other work has also shown that weight of gut contents is much higher when animals consume hay vs silage prepared from the same forage source. Our objective was to develop and evaluate a method to estimate weight of gut contents and use this estimate to convert body weight to empty-body weight. To achieve this objective a model was developed to predict weight of gut contents in cattle as a function of forage neutral detergent fiber, physical form of forage dry matter (hay vs silage and pasture), proportion of dietary concentrates and body weight.

Procedures

Experimental data were used to develop an equation to predict the fraction of body weight associated with gut contents, from the percentage neutral detergent fiber in the forage. Factors were then developed using data from other experiments to adjust this fraction for the effects of body weight, percentage of dietary concentrates and the physical form of forage dry matter. The adjusted gut contents fraction was then multiplied by body weight to obtain the weight of gut contents. This weight was subtracted from body weight to obtain empty-body weight. All body weights used in model development represented weight recorded early in the morning with animals having access to feed and water overnight. Hay and silage were the physical forms of forage dry matter used in the model. It was assumed that green pasture and dormant pasture were physically the same as silage or hay, respectively.

Data from 11 published experiments with 64 treatments (Table 1) were used to evaluate the model. Empty-body weight predictions obtained with the models used by the Agricultural Research Council (ARC) and the National Research Council (NRC) were also evaluated with these experimental data, and compared to the present model's predictions. The accuracy with which these three models (our present model, Agricultural Research Council, and National Research Council) predicted empty-body weight was evaluated by comparing observed to predicted values.

Results

The model to predict the weight of gut contents was:
weight of gut contents = Body weight x(53.54 + 3.29 x percentage neutral detergent fiber of forage) x (correction factor for body weight) x (correction factor for fraction of concentrates in diet) x (correction factor for forage physical form), where

correction factor for body weight = $(\text{body weight} / 200)^{-.332}$
correction factor for fraction of concentrates = $1 - .246 \times (\text{fraction of concentrates}) - 1.481 \times (\text{fraction of concentrates})^2 + 1.107 \times (\text{fraction of concentrates})^3$, and

correction factor for forage physical form was 1.35 for hays and 1 for silages.

Empty-body weight was calculated from the predicted weight of gut contents and the observed body weight. The model empty-body weight values calculated from predicted gut contents for the treatments using hay in Experiments 2, 4, and 5 were very different from observed values. In these experiments ammoniated stargrass and perennial ryegrass hay were used, and previous results have suggested that for ammoniated hays the correction factor for forage physical form should be 1. With this modification the calculated empty-body weight values using the present model predictions of gut contents were much closer to the observed values.

Observed empty-body weight is plotted in Figure 1, against the empty-body weight calculated with the present model, and empty-body weight predicted with the ARC and NRC models. For cases where the observed and predicted values are the same, then the points representing these paired values would lie on the 45 degree line shown in this figure. Points above the line mean that the predicted values underestimates the observed, and the opposite would be true for points below the line. Empty-body weight values calculated with the present model tended to be smaller than observed values for weights less than 400 lb. The method used by the ARC consistently overpredicted empty-body weight, and the NRC's method overpredicted empty-body weight for 50 of the 64 treatment means. These results confirm that the present model would be accurate in calculating empty-body weight from predicted weight of gut contents for weaned cattle, and suggest that it may not be appropriate between birth and weaning. This is understandable since these animals would be consuming milk, and their rumens have not been fully developed.

Referring to Figure 1, equations can be developed to adjust the empty-body weight predicted with the systems used by the ARC and NRC. It is possible that these adjusted predictions may be more accurate than the present model. These equations were developed, and the adjusted predictions of empty-body weight using the ARC and NRC models were compared to the present model's calculated empty-body weight values. The results of this analysis showed that the present model was still more accurate than the other two models.

[1]Williams and Keele are research animal scientists, Production Systems Research Unit, MARC; Waldo is a research animal scientist, Ruminant Nutrition Laboratory, Beltsville, MD.

The present model was developed with data on cool-season grasses, legumes and corn silage and it has not been fully tested with warm-season grasses, however, preliminary results with stargrass show no inconsistencies. Also it is possible that the correction factor for the fraction of dietary concentrates may not be appropriate in cases where very low-quality forages are supplemented with either cereals of high-protein byproducts, or protein supplements that differ in ruminal degradability. As more data become available, the model needs to be tested under these experimental conditions. Data used to develop and evaluate the model were obtained from animals that were on a specific plane of feeding for over three weeks, and model predictions of empty-body weight may not be accurate in the early period when animals are switched from restricted to full feeding or vice versa. Model inputs are dietary characteristics that can be obtained from routine forage analyses and unfasted body weight. This makes the model easy to use. It can be incorporated into diet formulation programs and systems models of cattle production.

Table 1—Summary of data from 64 treatments in 11 published experiments used to evaluate the model

Exp.	Number of treatments	Number of animals	Forage type	Neutral detergent fiber, %	Concentrate fraction in diet
1	3	54	Hay	40	.0
2	6	102	Hay	75-82	.0-.23
3	2	24	Silage	51.0	.38
4	8	48	Silage, hay & pasture	51	.0
5	12	66	Hay	66	.0-.44
6	4	24	Straw	80	.59-.87
7	4	40	Hay	42	.0
8	3	36	Hay	66	.6-.95
9	12	29	Straw	80	.83-.88
10	4	40	Silage	44-59	.0-.28
11	6	32	Silage	51.9	.0-.08

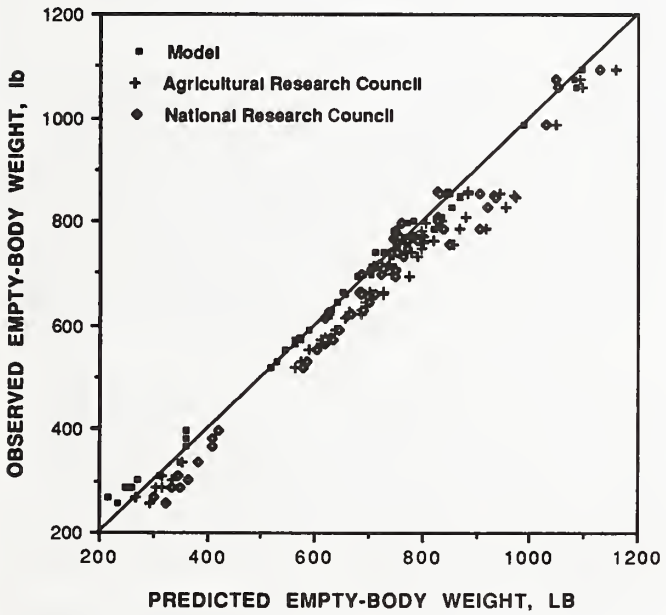

Figure 1 – Observed and predicted empty-body weight treatment means for 64 treatments in 11 published experiments.

Beef Cattle Salmonellosis: A Study of Oral *Salmonella typhimurium* and Topical *Salmonella newport* Inoculations

Ed K. Daniels, Neal E. Woollen, James S. Dickson, and E. Travis Littledike[1]

Introduction

Cattle are frequently infected with salmonellae by fecal-oral transmission or by being fed contaminated animal protein byproducts (40% are reported contaminated in the U.S.). Both could propagate salmonellosis in feedlots.

Research indicates that stress can induce shedding of salmonellae by asymptomatic carriers. Stress factors associated with salmonellosis include: transportation, starvation, changes in ration, overcrowding, age, pregnancy, parturition, exertion, anesthesia, surgery, intercurrent disease, and oral treatment with antibiotics and anthelmintics.

In this study, we have attempted to correlate dosage of *S. typhimurium* inoculum with disease, persistence of infection, and environmental contamination. The persistence and spread of *S. newport* placed on the skin of cattle was also studied.

Procedure

Inoculation procedures. Three groups of four steers each were inoculated orally with marker *S. typhimurium*. Groups 1, 2, and 3 were inoculated orally with 40,000,000, 7,000,000, and 1,000,000 units of *S. typhimurium*, respectively. The inoculum for each steer was placed in a gelatin capsule and administered with a balling gun. Ages of the steers were 19 mo (group 1), 8 mo (group 2), and 12 mo (group 3).

Group 2 steers were also inoculated topically with a strain of *S. newport*. Each hindfoot was placed in a plastic bag containing bovine feces inoculated with 1,100 salmonellae per lb.

Sampling procedures. Fecal samples were collected from the rectum and frozen at -4°F. At each sampling, rectal temperatures were recorded and observations of general appearance and clinical signs were noted.

In group 1, fecal samples were collected from two calves twice daily for 9 days after inoculation and then necropsied following euthanasia. The remaining two animals were sampled twice daily from 1 to 64 days, then once daily from 64 to 103 days, and, thereafter, once a day 3 days a wk (Monday, Wednesday, and Friday) from 103 to 365 days after inoculation.

In group 2, fecal samples were collected once daily for 39 days after inoculation and then once a day 3 days a wk to day 109. Rectal mucosa scrapings, using a wooden applicator stick, were collected from this group from day 5 to day 36 after inoculation. Microbiological samples of each foot were taken once a day (Monday through Friday) for 17 days after inoculation and then once a wk for two additional weeks. Foot samples were collected by scraping and swabbing the hoof walls with a sterile wooden applicator stick and then a piece of sterile gauze. On day 21 after inoculation, hair clippings from above the hoof were collected. Blood samples were taken for bacterial culture once a wk for 4 weeks.

In group 3, fecal samples were collected once daily for 43 days after inoculation and then once a day 3 days a wk to day 68.

Ground samples of the pens, as well as feed and water samples, were taken once during clinical signs for groups 1 and 3, and four times (once a wk for the first 4 wk) for group 2.

Tissue samples were harvested from all steers at necropsy following euthanasia. Sampling included brain, spinal cord, tonsil, various muscles, heart, lung, liver, spleen, kidney, urinary bladder, gall bladder, rumen (and contents), omasum (and contents), abomasum (and contents), duodenum (and contents), jejunum (and contents), cecum (and contents), colon (and contents), rectum (and contents), mesenteric lymph nodes, peritoneal fluid, pericardial fluid, and blood. Two steers in group 1 were necropsied 9 days after inoculation and the remaining two at approximately 1 year. Group 2 steers were necropsied 125 days after inoculation and group 3 steers were necropsied 80 days after inoculation. Tissue samples were frozen at -94°F.

Microbial analysis. Suspect colonies from all samples were identified by genus and species using a computerized microbiology system. *Salmonella typhimurium* isolates were also checked to verify compatibility with the marker inoculum. The salmonella isolates were also sent to the National Veterinary Services Laboratory for further verification.

Results

Group 1. Three steers showed severe clinical signs of diarrhea, elevated rectal temperatures (102 to 104°F), and ataxia 1 day after inoculation. The marker strain of *S. typhimurium* was found in fecal samples from two of the clinically ill steers 1 day after inoculation. The other clinically ill steer shed the marker bacteria on day 2. Fecal shedding of salmonellae persisted for 4 days in two of the steers and 6 days in the third. Clinical signs in two of the steers increased in severity and euthanasia was necessary on day 9. Salmonellae were never isolated from the feces of the steer showing no clinical signs.

Two steers were necropsied on day 9 and the marker strain of salmonella was found in the distal jejunum of both, in the proximal jejunum of one, and in the rectum of the other. *Salmonella infantis* was found in the urinary bladder, a mesenteric lymph node, and the caudal lumbar spinal cord of one steer. The other steer had *S. infantis* in contents of the middle jejunum, abomasal fluid, and the liver. This wild strain of salmonella was never isolated from fecal samples during the experiment.

Clinical signs of the surviving affected steer gradually decreased during the year; however, the steer developed signs of laminitis. Laminitis was not noted in any of the other steers (groups 1, 2, or 3). At necropsy, tissues and gastrointestinal contents of the two surviving steers were salmonella negative.

Group 2. Mild clinical signs of ataxia, slightly elevated rectal temperatures (102 to 103°F), and diarrhea were noted in all steers. Marker salmonellae were found in the fecal sample of one steer on day 4 after inoculation and in the fecal sample of another steer 13 days after inoculation.

[1]Daniels is a research microbiologist, Animal Health Systems Research Unit, MARC; Woollen is a veterinary medical officer, Animal Health Systems Research Unit, MARC; Dickson is a research food technologist, Meats Research Unit, MARC; and Littledike is the research leader, Animal Health Systems Research Unit, MARC.

These were the only positive fecal samples in this group. Salmonella contamination persisted between the claws for 8 days on one steer, for 3 days on another, and for 7 days on two steers. Attempts to isolate salmonellae by scraping the hoof wall or from clipped hair was unsuccessful. In all four animals, the marker strain of salmonella was found on one front foot and no positive ground samples were found.

Group 3. No clinical signs were observed. At necropsy 3 wk later, no gross lesions were noted and no salmonellae were isolated from tissues or gastrointestinal contents.

Conclusions

Severity of clinical signs was variable. Severity of disease appeared to be related to the infectious dosage, but individual variability was also observed.

Fecal shedding of salmonellae was also not consistent. Individual variability of both onset and duration was observed in groups 1 and 2. The fact that no fecal shedding of salmonellae was observed in group 3 suggests that there is a minimal infectious dose required to induce fecal shedding of salmonella. Long-term persistence of enteric *S. typhimurium* infection with recurrent shedding was not observed. A wild strain of salmonella, *S. infantis*, was recovered from various tissue samples of clinically ill steers, but never recovered from rectal fecal samples. This microorganism was not recovered from fecal samples of clinically normal pen-mates.

Ground, feed, and water samplings were not reliable in evaluating fecal shedding of *S. typhimurium* in the cattle pens. In spite of the spread of *S. newport* infection between the claws of the hindfeet to the forefeet, this microorganism was never recovered from ground samples. Even during periods of known fecal shedding, salmonellae could be recovered only from one sample of damp soil at the base of a watering unit.

It was demonstrated that active infection of the gastrointestinal tract can be present with no shedding of salmonellae in the feces. This observation suggests that isolation of salmonella from fecal material is a poor indicator of the salmonella infection status of beef cattle. Most of the time during clinical signs of salmonellosis, we were unable to isolate the organism in rectal samples or rectal mucosal scrapings. It was shown that even if fecal sampling is negative, carcass tissues may be contaminated with salmonellae and could possibly serve as a potential source of contamination to processing facilities, employees, and consumers.

Determination of Passive Immunity in Calves[1,2]

Louis J. Perino, R. James Sutherland, and Neal E. Woollen[3,4]

Introduction

Calves passively acquire a significant and vital portion of their immune protection from disease through consumption of the first milk (colostrum). The immunoglobulins (antibodies) that are contained in colostrum will help protect the calf from disease for the first several months of life. This process is called passive immunoglobulin transfer.

Failure of passive immunoglobulin transfer (FPT) is a serious and ongoing problem in calves. Although many factors that contribute to FPT have been examined, it continues to be an obstacle to profitability. Calves that do not receive adequate colostrum are at increased risk of infection from a variety of disease-causing organisms.

Several methods of detecting FPT have been described. Evaluating the status of passive immunity in calves is hindered by deficiencies in the available testing technologies. The most accurate means to assess FPT is determining concentrations of serum immunoglobulin. The predominant type of immunoglobulin transferred from the cow to the calf through colostrum is immunoglobulin G (IgG). Direct measurement of serum concentrations of IgG is usually accomplished using radial immunodiffusion. The value of this test is limited by the high cost involved, the technical expertise required, and the lack of relevance of the test results after the 24 to 48 hr required for the test to run.

Several indirect methods of determination are available. These include zinc sulfate turbidity, sodium sulphite precipitation, glutaraldehyde coagulation, and serum refractometry. These are indirect measurements of the immunoglobulin levels of the calf and therefore are subject to artifactual readings due to aberrations in hydration status, total blood protein levels, and other blood attributes. Some of the above tests (zinc sulfate turbidity, sodium sulphite precipitation, and glutaraldehyde coagulation) require the transport of test tubes and reagents to the field. These three tests are semiquantitative and provide estimates of minimal levels or ranges of serum immunoglobulin levels. Refractometry is simple, quick, and inexpensive, but considered the most inaccurate estimator of immunoglobulin status.

Gamma-glutamyltransferase (gamma-GT) is a membrane associated enzyme located in multiple sites throughout the body. Gamma-GT is located primarily in cells that have absorptive or secretory functions. Serum level of gamma-GT is recognized as a useful clinical indicator of liver disorders in many species. Activity of gamma-GT in colostrum has been reported to be high in a number of species, including dogs, sheep, cattle, and human beings. In many of these species, serum activity of gamma-GT in neonates that have consumed colostrum is elevated. However, this is not true in all species, with horses being a reported exception.

The purposes of this study were to characterize the activity of serum gamma-GT in newborn calves before and after suckling and to explore the usefulness of serum gamma-GT as an indicator of FPT in calves.

Procedure

Blood samples were collected from the calves of 48 four-breed composite heifers (1/4 Red Poll, 1/4 Hereford, 1/4 Pinzgauer, 1/4 Angus) at the time of birth and at 1 day of age. Serum was harvested from the blood, frozen, and stored for later assay.

At birth, calves received an ear tag, oral rotavirus and coronavirus vaccine, and their navels were treated with iodine. At approximately 60 days of age, and 3 wk before weaning (approximately 5 mo of age), the calves were vaccinated with multivalent clostridial and leptospiral vaccines. A modified-live virus vaccine containing infectious bovine rhinotracheitis and bovine virus diarrhea viruses was also given 3 wk before weaning.

Health status of the calves and cause of morbidity were determined by trained animal caretakers under veterinary supervision. Unusual cases were referred to the veterinary staff for diagnosis.

Serum concentrations of IgG were determined using a commercial radial immunodiffusion kit (VMRD RID Kits, VMRD, Pullman, Washington). The upper and lower limits of detection were 3,300 and 412 mg/dl, respectively. Serum total protein values were assessed with a refractometer. Activity of gamma-GT in serum was measured by automated spectrophotometry using a commercially available kit (gamma-GT reagent 44074, Ciba-Corning Diagnostics Corp, Oberlin, Ohio).

Correlation coefficient, means, percentages, and standard deviations[5] were generated with a commercial microcomputer spreadsheet program (Lotus Development Corp, Cambridge, Massachusetts). Mantel-Haenszel Chi-squares, relative risk, and Kappa values were calculated using a public-domain microcomputer epidemiologic statistic program (USD Inc, Stone Mountain, Georgia).

Results

Paired serum samples were obtained from 48 calves. Activity of gamma-GT was elevated in calves that suckled colostrum. The degree of elevation was proportional to the amount of colostrum consumed, as indirectly indicated by serum concentrations of IgG. Calves suckling colostrum had 10.0 and 1.3 times greater serum concentrations of IgG and protein, respectively, and a 26 times greater serum activity of gamma-GT, compared to concentrations at birth. At birth the avg serum concentrations of IgG and protein were 131 mg/dl[6] and 3.9 g/dl, respectively, and serum activity of gamma-GT was 28 IU/L. After 24 hr these values had increased to 1,400 mg/dl,[6] 5.0 g/dl, and 734 IU/L, for the same respective parameters.

Calves were classified as having FPT, PFPT, and normal passive transfer, on the basis of concentration of serum

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[1]The full report of this research is accepted for publication in American Journal of Veterinary Research as journal series article number 9837 of the University of Nebraska-Lincoln Agricultural Research Division.

[2]Published as a research bulletin of the University of Nebraska-Lincoln Agricultural Research Division.

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[4]Appreciation is expressed to the cattle operations staff, W. Gordon Hays, manager, for assistance in collection of samples and to Jill Boyum and Tammy Sorenson for technical assistance.

[5]Standard deviations were corrected to provide unbiased estimates of standard deviation.

[6]Averages at birth and 24 hr include 14 and 8 calves, respectively, with serum IgG concentrations below 412 mg/dl for which 411 mg/dl was used to determine the mean.

IgG detected by radial immunodiffusion. Twenty-one percent of calves had FPT (Table 1).

Serum IgG concentrations, serum protein concentrations, and serum activity of gamma-GT were related (Figure 1). The correlation coefficient between IgG and gamma-GT was 0.41. The correlation coefficient between IgG and protein was 0.77.

Significant differences were detected in the morbidity between calves classified as having FPT, PFPT, and normal passive transfer (Table 2). The calves with FPT had a 9.5 times greater risk of becoming classified as sick prior to weaning compared with calves with PFPT and normal passive transfer (P=0.0004). The causes of morbidity were variable (Table 2), suggesting a generalized immunodeficiency.

The sensitivity and specificity of a cut-off value of 200 IU gamma-GT/L serum for diagnosing FPT were 80% and 97%, respectively. The sensitivity and specificity of a cut-off value of 4.2 g protein/dl serum for diagnosing FPT were 80% and 100%, respectively. The Kappa values for diagnosis of FPT using serum concentrations of IgG versus serum activity of gamma-GT, IgG versus protein, and gamma-GT versus protein were 0.72, 0.86, and 0.79, respectively.

In summary, serum activity of gamma-GT is elevated in 24 hr old calves that have consumed colostrum; therefore diagnostic value of elevations of gamma-GT for hepatic pathology is limited during at least the first wk of life for a calf that has received an adequate amount of colostrum.

The least expensive and most rapid indicator of passive immune status in this study was determination of concentration of serum total protein. However, refractometric total serum protein can be misleading as other plasma analytes such as glucose, urea, and creatinine contribute to the refractive index. Thus, sick and/or dehydrated calves can render spuriously high total serum protein values.

Table 1—Number of calves and avg serum IgG, gamma-glutamyltransferase (gamma-GT), and total protein (TSP) values at 24 hr after birth for calves classified as failure of passive transfer (FPT), partial failure of passive transfer (PFPT), and normal

Classification serum IgG levels	FPT <800 mg/dl	PFPT 800-1,600 mg/dl	Normal >1,600 mg/dl
Total calves	10	18	20
Avg IgG mg/dl	449.0[a]	1,272.0	1,990.0
Avg gamma-GT IU/L	154.0	706.0	1,049.0
Avg TSP g/dl	4.0	5.0	5.5

[a]Includes eight calves with IgG concentrations below 412 mg/dl for which 411 mg/dl was used to determine the mean.

Table 2—Clinical diagnoses of sick calves classified as failure of passive transfer (FPT), partial failure of passive transfer (PFPT), and normal at 24 hr after birth

Classification serum IgG levels	FPT <800 mg/dl	PFPT 800-1,600 mg/dl	Normal >1,600 mg/dl
DIAGNOSIS:			
Diarrhea	2	0	1
Keratoconjunctivitis	1	0	0
Arthritis	1	0	0
Pneumonia	1	0	0
Omphalophlebitis	0	0	1
TOTAL SICK	5[a]	0	2
TOTAL AT RISK	10	18	20

[a] Values differ from other values in row (P<.05).

Serum activity of gamma-GT also gave reliable indications of concentration of passive immunity but such determinations were more costly and time consuming to determine than those used for serum protein. Serum activity of gamma-GT is not susceptible to changes in other serum analytes and is less susceptible to artifacts caused by dehydration.

Determination of either gamma-GT serum activity or protein serum concentration was less expensive and gave results sooner than radial immunodiffusion for IgG. Determination of both would be useful in determining the success or failure of colostral management in groups of bovine neonates. The value in applying these tests lies in evaluation of groups of calves. Failure of passive transfer is a management problem and the prevalence of subsequent infection depends largely on the success of the colostral management. The role of these tests lies in testing healthy calves in the range of one to seven days of life. A minimum of ten calves should be sampled since the greater the sample size the less sensitive and more specific a test can afford to be.

Once effective methods of identifying calves that have experienced failure of immunoglobulin transfer have been validated, cattle producers can use these methods as management tools. If too many calves are found to have experienced failure of immunoglobulin transfer, producers can alter their management. Individual calves that have experienced failure of immunoglobulin transfer can receive special treatments such as supplementary colostrum and additional vaccinations. Evaluation of the efficacy and cost effectiveness of such interventions are part of the ongoing research in this project.

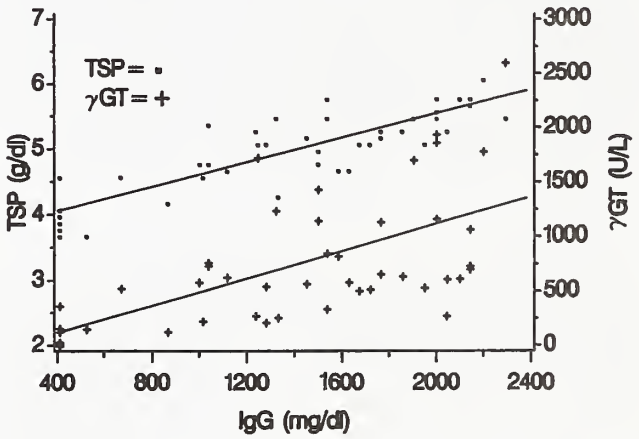

Figure 1 – Serum IgG concentrations vs serum protein concentrations (TSP) or serum activity of gamma-glutamyltransferase (gamma-GT) for calves 24 hr after birth.

Development of a Method for the Serological Differentiation Between Animals Either Vaccinated with Killed Virus Vaccine or Infected by Bovine Viral Diarrhea Virus (BVDV)

Jimmy Kwang and E. Travis Littledike[1]

Introduction

Bovine viral diarrhea (BVD), caused by the BVD virus, has been recognized in many parts of the world and is considered to have a marked economic impact on the cattle industry. In the U.S. alone, serological surveys indicated that 60 to 80% of the cattle population have antibodies to the virus. There are many strains of bovine viral diarrhea virus (BVDV) which differ in their ability to cause changes in cell culture. Thus, cytopathic and noncytopathic biotypes of BVDV are identified. The cytopathic strains induce a vacuolation of the infected cultured cells, where noncytopathic strains do not.

Control of BVDV has been attempted for many years by use of either modified-live or killed virus vaccines. The killed virus vaccine is more commonly used. The modified-live virus vaccine is known to cause complications during pregnancy, potentially fetopathogenic effects being a major concern. There is evidence that vaccination of persistently infected cattle with modified-live virus vaccine can result in severe mucosal disease. Due to the ubiquitous nature of BVDV, producers may find advantages in designing BVD control procedures for a herd to be able to differentiate between cattle that 1) have received modified-live BVD virus vaccination, 2) have received killed BVD virus vaccination, or 3) were naturally infected. This study explored the potential of BVDV protein, p80, to allow differentiation of the above three conditions.

The genome of an isolate of a BVDV strain has been sequenced. Encoded within the genome are at least four primary gene products (proteins): p20, gp116, p125, and p133. There is evidence that p125 polypeptide precursor gives rise to p80 polypeptide due to the breakdown of this precursor protein in cells infected with BVDV. The p80 area of the BVDV genome is well conserved in the many BVDV strains that have been isolated.

Procedure

New molecular biology techniques permitted the manipulation of the BVDV genome to allow isolation and recombination of the p80 gene into a DNA structure designed for production of the p80 specific protein. The recombinant p80 protein was produced, expressed, and purified in sufficient quantities to develop a specific immuno-blot assay for BVDV antibodies in cattle sera. Twenty-four cattle sera were tested: eight of the cattle were vaccinated with modified-live virus vaccine, eight were vaccinated with killed virus vaccine, and eight were naturally infected with BVD.

Results

The immuno-blot assay that was developed was tested for BVDV-p80 antigen-antibody reaction. When the BVDV-p80 protein was reacted with the cattle sera in the immuno-blot test, the following results were obtained: all sera from modified-live virus vaccinated cattle were positive; all sera from killed virus vaccinated cattle were negative, and all sera from naturally infected cattle were positive (Table 1). Since p80 is not a structural protein of the virus and the killed virus doesn't replicate in the host cell, there is not sufficient p80 antigen in cattle vaccinated with killed BVD vaccine to cause production of p80 antibodies in cattle. Therefore, cattle vaccinated with the killed BVD vaccine either do not make antibodies or do not produce detectable levels of antibodies to the p80 region of the virus. Thus, the cattle test negative. However, modified-live virus used for vaccination replicated in the host cell after vaccination and produced large amounts of p80; therefore, antibodies were produced in the cattle to the p80 and the cattle tested positive.

The significance of this finding is two-fold. First, p80 is capable of differentiating between the modified-live and killed virus vaccines. Second, if a herd was vaccinated only with killed virus vaccine and tested with p80 immuno assay, the expected results would be negative—if the herd was clean of BVDV. Any positive results would indicate that a source of natural infection must be present in the herd. This source could be from persistently viremic BVD carriers, that may or may not exhibit signs of the infection, but would spread BVD throughout the herd and give birth to carrier calves which would continue the spread of BVD in the herd. To ultimately control BVDV, carriers of the BVD virus must be identified and eliminated from the herd and no new carrier cattle added. In addition, effective vaccination of the cattle before breeding would protect the fetal calves from becoming carriers and perpetuating BVD in the herd.

Table 1—Comparison of BVDV p80 antibody response in cattle vaccinated with either MLVV, KVV, or NI with virus

Animal no.	BVDV exposure	Serum neutralization test	p80 immuno-blot assay
1	MLVV	32	+
2		16	+
3		64	+
4		16	+
5		32	+
6		32	+
7		32	+
8		32	+
9	KVV	4	-
10		8	-
11		4	-
12		<2	-
13		<2	-
14		<2	-
15		4	-
16		8	-
17	NI	>256	+
18		>256	+
19		>256	+
20		>256	+
21		128	+
22		>256	+
23		>256	+
24		128	+

BVDV = Bovine viral diarrhea virus.
MLVV = Modified-live virus vaccine.
KVV = Killed virus vaccine.
NI = Naturally infected.

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Development of a Sensitive Antibody Detection Method to Bovine Viral Diarrhea Virus (BVDV) Infection

Jimmy Kwang and E. Travis Littledike[1]

Introduction

Bovine viral diarrhea virus (BVDV) is a RNA virus and a prototype member of the pestivirus genus in the genetic family Flaviviridae. Due to the rapid growth of BVDV molecular biology in the last 4 yr, our understanding of the genomic organization of BVDV has greatly increased, and a protein encoding map of the BVDV genome has been established. According to this map, it is generally accepted that a glycoprotein, identified as gp116, is the precursor which gives rise to gp62 and gp53 proteins through a proteolytic process. Further protein break down of gp62 yields glycoproteins gp48 and gp25. The order of these genes in the BVDV genome would thus be gp48-gp25-gp53. Based on the serological testing results of cattle to individual BVDV proteins, a strong immune response to glycoproteins gp53 and gp48 has been found.

Although considerable literature exists on the diagnosis of BVD disease, the methodologies (virus isolation, serum neutralization, etc.) are either time consuming, expensive, inconsistent, or unsuitable for use in large populations of cattle. Recently, recombinant techniques have found wide application in a second-generation assay for the detection of viral disease infection. We have produced recombinant gp48 in large amounts using these recombinant techniques. The large-scale production of the recombinant gp48 protein provided a convenient and economical source of immunobiologically useful material. This recombinant protein demonstrated great sensitivity and specificity for BVD antibody detection.

Procedure

A total of 175 bovine sera were included in this study. Eighty samples were from MARC, where a BVDV vaccination program has been practiced with both killed and modified-live virus vaccines during the last 10 years. The remaining 95 samples were from an isolated commercial ranch in north-central Nebraska that had never been vaccinated for BVDV. Therefore, antibody-positive individuals may represent the outcome of either, or various combinations of, natural infection, killed virus vaccine, modified-live virus vaccine, or passive immunity from the mother. In addition, five serum samples were used as the negative control and two cattle sera immunized with BVDV previously were used for the positive control.

To allow study of the role of BVDV-gp48 during infection and the subsequent immune response in cattle, we developed a specific immune assay (immuno-blot assay) by incorporating the recombinant gp48 protein into the test.

Results

The serum neutralization tests identified the 175 bovine samples as follows: 89.1% (156/175) of the sera had antibodies to BVDV and 10.9% (19/175) did not. When subjected to the recombinant-gp48 immuno-blot assay, sera from the 156 animals with serum neutralization titer equal to or greater than 1:4 reacted positively, indicating a gp48 antigen-antibody reaction was present. The 19 sera that did not have antibodies to BVD (serum neutralization titer less than 1:4) were negative in the immuno-blot. To verify the 19 negative sera samples did not have gp48 antibodies, a third test (radioimmunoprecipitation) was performed. The results of these tests were in complete agreement with the serum neutralization and immuno-blot assays. The gp48 protein was readily recognized by the BVDV-gp48 positive serum and the five negative control sera showed no reactivity. Thus, the gp48 recombinant protein proved to be both specific and sensitive when used in this immuno-blot assay.

To determine if the antibody response to gp48 differs in cattle with 1) natural infection, 2) killed virus vaccine, or 3) modified-live virus vaccine, we used three pair of sera from calves before and 4 wk after exposure to these treatments. Their serum neutralization titers and immuno-blot reactivity are shown in Table 1. This information shows the calves developed an immune response to gp48 following exposure to all three treatments. This further indicated that gp48 is a major structural protein of BVDV.

Progress in understanding the molecular biology of BVDV and the role of individual proteins in infection and immunity has been slow. Procedures have only recently been developed which increase the ease and efficiency of producing such proteins in quantity and quality to allow in-depth study. Bovine viral diarrhea virus-gp48 can be readily produced by the method described in this report and it can be used in an immuno-blot assay to detect the presence of antibodies produced by natural infection and vaccination with killed or modified BVD virus. Bovine viral diarrhea virus-gp48 is a highly conserved and recognized gene across the spectrum of BVDV strains tested. Therefore, the use of BVDV-gp48 recombinant protein may prove to be a strong candidate for developing a BVDV antibody detection test kit. The rapid, sensitive nature of such a test, and its low cost, could prove to be an effective tool for diagnosis and control of BVD in large and small cattle herds.

Table 1— Pair serum samples examined for antibody reactivity before and after different forms of virus exposure

Pair	Sera	Form of exposure	SN titer	Immuno-blot assay
1	a		<2	-
	b	NI	256	+++
2	a		<2	-
	b	MLVV	64	+++
3	a		<2	-
	b	KVV	16	++

SN = Serum neutralization.
a = Before exposure.
b = After exposure.
NI = Natural infection.
KVV = Killed virus vaccine.
MLVV = Modified-live virus vaccine.

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Consequences of Antigenic Diversity of Bovine Viral Diarrhea Virus

E. Travis Littledike, Steven R. Bolin, and Julia F. Ridpath[1,2]

Introduction

Two main biotypes of bovine viral diarrhea virus (BVDV) have been identified based on their ability to cause changes in tissue culture cells. The cytopathic biotype multiplies in tissue culture and kills cells, while the noncytopathic biotype slowly multiplies in tissue culture and has much less ability to kill tissue culture cells. In general, cytopathic BVDV biotypes cause acute infections that often kill the bovine fetus, while noncytopathic BVDV biotypes often result in chronic infection of the fetus which, subsequently, develops in calves and adults that carry and shed noncytopathic viruses at high levels for the rest of their lives.

Previous studies have indicated that cytopathic and noncytopathic viruses are antigenically similar. Also, after vaccination of cattle with modified-live or killed BVDV vaccines, antibodies are induced that neutralize a broad range of BVDV. However, very significant antigenic diversity among BVDV has been described. Also, studies indicated that some neutralizing antibodies from cattle that have recovered from BVDV react differently with several BVD isolates. In addition, monoclonal antibodies developed against specific BVDV isolates can differentiate BVDV into several groups and, when cattle which are persistently infected with noncytopathic BVDV are challenged with cytopathic BVDV, the antibodies they produce have a very narrow range of viral neutralizing activity.

Thus, some antigenic diversity among BVDV, as detected by neutralization tests, is well established. However, there is little information that shows the practical consequences of this antigenic diversity relative to the disease in cattle.

The primary purpose of this study was to identify cattle in MARC's herd that were persistently infected with BVDV and test the isolates of BVDV from the MARC herd to determine if these natural field viruses could be neutralized by serum obtained from MARC cows vaccinated with killed BVDV.

Procedure

Source of sera. Serum was obtained over a 5-wk period in the fall of 1988 from 5,726 cows maintained as a semi-closed herd on pasture. At that time, the herd had been on a killed virus vaccination program for BVD for more than 7 years. The vaccine used was of bovine-cell origin and contained the Singer isolate of cytopathic BVDV. The vaccination program consisted of calfhood vaccination with the first dose given 1 mo before weaning and the second dose given 4 wk later at the time of weaning. Thereafter, cows were revaccinated with a single dose of vaccine 2 to 3 wk before breeding. Approximately, 80% of cows calved in April and May and the remaining 20% calved in August and September. At the time samples of serum were obtained, cows calving in the spring were at approximately 3 mo of gestation and had been vaccinated approximately 4 mo previously. Cows that calved in the late summer had calved approximately 2 mo previously and were vaccinated approximately 12 mo previously. The herd was managed as several groups of varying numbers maintained on separate pastures. At weaning, calves from all groups were moved to a feedlot. Performance of a calf in the feedlot was one of the criteria used to select herd replacements and cull dams. The replacement rate for the herd was approximately 20% per year.

Viral neutralization tests. Sera were tested for neutralizing antibodies against one or more of the following viruses: cytopathic viral isolates BVD-TGAC and BVD-Singer, and noncytopathic viral isolates BVD-3659, BVD-2541, BVD-9789, BVD-NEB, BVD-7443, BVD-639, and BVD-VM. Noncytopathic viruses 9789, NEB, 7443, and VM were isolated from persistently infected cattle, BVD-639 was isolated from the uterus of a cow that aborted, and BVD-3659 and BVD-2541 were isolated from persistently infected cattle identified during this study. With the exception of BVD-3659 and BVD-2541, the viruses used were antigenically distinct from each other when tested against a panel of monoclonal antibodies that had neutralizing activities. Viruses 3659 and 2541 were antigenically similar to each other, but distinct from the other viruses.

Neutralization tests against BVD-TGAC virus were performed on all samples of serum. Those samples of serum that had neutralized antibody titers of less than 2, 2, 4, or 8 were tested for neutralizing antibody titer against BVD-Singer virus. In addition, 18 selected samples of serum that had neutralized antibody titers of less than 2, 2, or 4 against BVD-TGAC virus were tested for neutralizing antibodies against the aforementioned seven noncytopathic BVD viruses. All samples of serum (n=56) that had neutralized antibody titers of eight against BVD-TGAC virus were tested for neutralizing antibodies against noncytopathic BVD-3659 virus.

Immunoprecipitation. In selected samples of serum from killed virus vaccinates that had neutralized antibody titers of less than 2 to 256 against BVD-TGAC virus, antibody specificity for polypeptides induced by BVD-Singer virus (vaccine virus) was identified by immunoprecipitation. For comparison, viral induced polypeptides were immunoprecipitated with samples of serum obtained from modified-live virus vaccinates that had neutralizing titers of 2 to 16 against BVD-TGAC virus.

Results

Virus was isolated from 3 of 448 samples of serum that had neutralized antibody titers of 64 or less against BVD-TGAC virus (Table 1). In those three samples of serum, the neutralizing antibody titers against BVD-TGAC virus were less than 2, 2, and 32. The corresponding neutralizing antibody titers against BVD-Singer virus were 32, 64, and 256. Persistent infection was subsequently confirmed in two cows (ages 2 and 3 yr) by isolation of virus (designated BVD-3659 and BVD-2541) from a second sample of serum obtained at least 4 wk before or after the original sample of serum. Due to poor performance, a third cow (2 yr of age) had been sold soon after the original sample of serum was obtained. It was not possible to confirm persistent infection in that cow. Virus was not isolated from sera obtained from siblings of one persistently infected cow or the dam of the other persistently infected cow.

Neutralizing antibody titers of four or less against BVD-TGAC virus were detected in samples of serum obtained

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[2]The full report of this work was published in Am. J. Vet. Res. 52(7):1033-1037.

from 91 and 40 cows that calved in the fall and spring, respectively. In each of those 131 samples of serum, neutralizing antibody titers against BVD-Singer virus were greater than the corresponding neutralizing antibody titers against BVD-TGAC virus (Table 1). From those 131 samples of serum, 18 were selected and further tested for neutralizing antibodies against seven noncytopathic BVD viruses. None of these 18 samples of serum contained neutralizing antibodies against all seven noncytopathic viruses (Table 2). Noncytopathic BVD-3659 virus was not neutralized by any of the selected samples of serum. The BVD-3659 virus was neutralized by 18 of 56 samples of serum that had neutralized antibody titers of eight against BVD-TGAC virus (data not shown).

Conclusion

Although the herd surveyed in this study had been on a killed BVDV vaccination program for 7 yr, two persistently infected cows were identified. Persistent BVDV infection is lifelong and occurs in calves born to dams that have an acute, transient viral infection during the first 4 mo of gestation or to dams that are themselves persistently infected. The two persistently infected cows likely represented failure of vaccination to protect against fetal infection under natural conditions. That finding supported previous studies in which experimental, killed BVDV vaccines failed to prevent transplacental transmission of virus in challenged, exposed cows.

Failure of vaccination to protect the fetus might be explained by antigenic differences among BVD viruses. In several sera from this herd, the titer of neutralizing antibodies against the vaccine virus was relatively high (64 to 256); however, several isolates of BVD virus were identified that escaped neutralization by those same sera. Those data clearly indicate antigenic diversity among BVD viruses. Included among the viruses that escaped neutralization were noncytopathic viruses BVD-3659 and BVD-2541 that were isolated from persistently infected cows in this herd. Thus, the persistently infected cows likely represented a practical consequence of antigenic diversity among BVD viruses.

Natural decay of viral-specific antibody likely contributed to the lack of detectable antibodies against certain BVD viruses. Data from this study support this hypothesis. Approximately, 80% of the cows were given a booster dose of vaccine 4 mo before samples of sera were obtained and the remaining 20% of cows were boostered with vaccine 12 mo before sampling. A disproportionately high 91 of 131 samples of serum (70%) that had neutralizing antibody titers of four or less against BVD-TGAC virus were obtained from cows vaccinated 12 mo before sampling. Thus, failure to detect neutralizing antibodies against certain BVD viruses several mo after vaccination may have been attributable to viral antigenic diversity and natural decay of antibodies.

On the basis of the large number of samples of sera that had high titers of neutralizing antibodies against a BVD virus antigenically distinct from the killed vaccine virus, and on the pattern of immunoprecipitated viral-induced polypeptides associated with those sera, we speculate that most of the cattle in this herd had been infected with BVD virus. Identification of only two persistently infected cows might seem trivial; however, the rate of persistent infection probably would have been higher if newborn calves were tested instead of cows. Subsequent to completion of this study, eight calves in this herd (approximately 2 yr old) were identified as persistently infected with BVD virus. The persistently infected cattle in this herd likely were born to vaccinated cows that were infected with field virus during early gestation. Consequences of antigenic diversity among BVD viruses are not likely limited to fetal infections in vaccinated dams. Newborn calves with colostral antibody or vaccinated feedlot calves might be susceptible to disease induced by certain antigenic variants of BVD virus.

Table 1—Distribution of neutralizing antibody titers ($-\log_2$) against bovine viral diarrhea (BVD)-TGAC virus in all samples of serum and range of titers and geometric mean titers of neutralizing antibodies against BVD-Singer virus in samples of serum that had neutralized antibody titers of three ($-\log_2$) or less against BVD-TGAC virus

Number of sera	Neutralizing antibody titer to TGAC virus	Geometric mean titers to Singer virus	Range of neutralizing antibody titers to Singer virus
48	0	4.09	1 to 8
42	1	5.25	3 to 8
41	2	6.11	3 to 8
56	3	5.21	3 to 8
61	4	ND	ND
70	5	ND	ND
130	6	ND	ND
253	7	ND	ND
5,025	8	ND	ND

ND = Not done.

Table 2—Neutralizing antibody titers (-log2) in select sera against bovine viral diarrhea (BVD)-TGAC virus, corresponding titers of neutralizing antibodies against BVD-Singer virus, and presence (+) or absence (-) of detectable concentrations of neutralizing antibodies against seven antigenically distinct noncytopathic BVD viruses

Neutralizing antibody titer		Neutralizing activity against noncytopathic viruses[a]						
TGAC virus	Singer virus	3659[b]	2541[b]	9789	NEB	7443	639	VM
0	1	-	-	-	-	-	-	-
0	1	-	-	-	-	+	+	-
0	3	-	-	-	-	-	+	-
0	4	-	-	-	-	-	-	-
0	6	-	-	-	-	-	-	-
0	6	-	-	-	-	+	+	+
0	7	-	-	-	-	-	-	+
0	7	-	-	-	+	+	-	+
0	8	-	-	-	-	+	-	-
1	7	-	-	-	+	+	+	+
1	7	-	+	+	+	+	+	+
1	8	-	-	-	-	-	+	+
1	8	-	-	-	+	+	+	+
2	3	-	-	-	-	-	-	+
2	6	-	+	+	+	+	+	+
2	8	-	-	-	+	+	+	+
2	8	-	-	+	+	+	+	+
2	8	-	+	-	+	+	+	+

[a] Serum was diluted 1:1 with fluid containing virus.

[b] Viral isolates from MARC herd.

Brachygnathia in Simmental Cattle

Neal E. Woollen[1]

Introduction

Brachygnathia is a deficit in mandibular length causing the incisor teeth to meet the upper dental pad behind its anterior angle. It is a problem to breeders of both red and black Simmental cattle, as well as other breeds. The condition has been considered inherited as a simple autosomal recessive trait. It has also been observed as one part of a lethal, multiple-defect syndrome in Simmentals caused by the calf being born with an extra chromosome (Trisomy 17). Intrauterine infection with bovine viral diarrhea-mucosal disease virus (BVD-MD) also can cause the defect, but usually in this case the calf is also born with a variety of additional problems. In Angus cattle, the defect has also been observed accompanying osteopetrosis, an inherited bone defect.

Selective culling and breeding practices designed to remove an undesirable genetic trait have been unsuccessful for a number of producers of both red and black Simmentals. For that reason, we have been studying the inheritance of this condition in more detail.

Procedure

An affected and distantly related red bull and heifer were selected as the foundation for this project. Following superovulation and embryo transfer, 14 calves were produced from this mating. One affected heifer was selected to mate back with her sire. Eleven calves have been produced from this mating. The same bull was mated to an affected Angus cow. Six calves have been produced from this mating. Semen has been collected from a black Simmental bull. To evaluate the source of deleterious genes in black Simmental cattle, he will be mated to the same females.

Results

The initial mating produced three (21%) affected calves. The father- daughter mating produced two (18%) affected calves. The Angus-Simmental mating has produced no affected calves. Affected calves have had no additional significant defects, and have been of both sexes.

In a recessive mode of inheritance, two affected cattle should produce 100% affected calves if penetrance is complete. It is clear that there are significant factors affecting penetrance, or that the condition is not due to recessive gene action.

There is no significant difference in the percentage of affected calves produced from either Simmental mating. It is reasonable to assume that if enough calves were produced, the affected percentage would be roughly 25%. This pattern of inheritance is compatible with overdominance at two gene loci (locations) involving four genes. In overdominant inheritance, the heterozygote (Aa) is different from either the homozygous dominant (AA) or recessive (aa). Overdominance at one locus would produce 50% affected calves, and overdominance at two loci would produce 25% affected calves.

The fact that no affected calves have been produced from the Simmental-Angus mating suggests that inheritance of the condition is different between the two breeds. However, with only six calves produced to date, this is only an assumption. If this pattern continues, we also can assume the condition in black Simmentals is of either Simmental or Angus origin and not combined genetic action. Identical matings using the black Simmental bull should clarify this matter.

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Transmission of Bovine Leukosis Virus[1]

Louis J. Perino, E. Travis Littledike, Larry V. Cundiff, and Catherine E. Dewey [2,3,4]

Introduction

Bovine leukosis virus is an exogenous retrovirus (Retroviridae, Oncovirinae) that infects lymphocytes of cattle. Infection with bovine leukosis virus and the concomitant antibody response are lifelong. Infection can result in several outcomes, including production of antibodies against bovine leukosis virus without other evidence of infection, inversion of the T:B lymphocyte ratio, persistent lymphocytosis, and clinical lymphosarcoma.

The prevalence of an infection in a population of animals is the proportion of the group infected at any given time. Surveys have shown the prevalence of bovine leukosis virus infection in cattle populations ranging from 0 to nearly 100%. This wide range of prevalence levels is likely due to variations in risk factors such as husbandry practices, insect vectors, and genetic resistance. For example, prevalence tends to be higher in dairy than beef cattle and in cattle in Southern vs Northern states.

The relative importance of the known modes of transmission of bovine leukosis virus has not been established in beef cattle. Also, the economic impact of bovine leukosis virus infection in beef cattle has not been examined. However, the presence of cattle infected with bovine leukosis virus in a herd drastically reduces opportunities to export cattle and/or semen to many countries.

Excluding an early transient viremia, the virus locates in lymphocytes as a DNA provirus. Because of its cell-associated nature, transmission is believed to occur by movement of infected lymphocytes to susceptible animals. Intradermal, subcutaneous, intramuscular, or intravenous inoculation of as little as one microliter of blood or intracutaneous inoculation of 2,500 lymphocytes from an infected animal (equivalent to .5 microliter of blood) results in transmission of bovine leukosis virus.

Transmission of an infectious agent in this manner is a form of horizontal transmission. Other means of horizontal transmission have been investigated, including casual contact in common housing; animal husbandry procedures such as dehorning without sanitizing the dehorner between cattle, tattooing with common pliers, rectal palpations with common sleeves, and injections with common needles; and blood feeding arthropods. In addition, transmission from the dam to calf, termed vertical transmission, has also been shown to occur with bovine leukosis virus.

The purpose of these studies was threefold: 1) to characterize the bovine leukosis virus status of the MARC cattle population, 2) to investigate the extent and significance of vertical transmission of bovine leukosis virus in the MARC cow herd, and 3) to investigate the role of specific management practices in horizontal transmission of bovine leukosis virus in the MARC cattle herd.

Procedure

Bovine leukosis virus infection detection. Bovine leukosis virus infection status was assessed by the presence of serum antibodies against bovine leukosis virus. Blood samples were collected from cows by jugular or coccygeal venipuncture. Serum was harvested from the blood, frozen, and stored for later testing. Serum antibodies to bovine leukosis virus were detected using agar gel immunodiffusion.

Phase I. A sample size was determined for each area of the MARC that was large enough to detect at least one positive animal with 95% confidence if infection rates were at or above 5% in a group (Figure 1). A random sample of the 1989 adult cattle at each area was identified. The 1989 sera collected from these cattle were retrieved and the bovine leukosis virus infection status was determined. All adult cattle in the twinning project (area 52 and 391 of 821 head at area 73) were tested because of a previous history of bovine leukosis virus infection followed by an eradication program.

Based on results of this survey and analysis of cattle movement patterns, all adult cattle from areas 12, 25, 58, 67, and 73 (non-twinning project) were tested. In addition all cattle in the disease resistance herd (area 18) and all area 18 Angus were tested.

The serum collected in 1988 was tested from any cow found positive in 1989. Sera from all available dams and progeny of infected cattle were also tested.

Phase II. Area 12, having been determined to have the largest number of bovine leukosis virus infected cows, was selected for more intensive monitoring. All positive cows were sampled for hematologic determination of peripheral lymphocyte numbers and T:B lymphocyte ratios. The order in which cows were processed was recorded. Processing activities included injection with common needles, rectal palpation with common sleeves, replacement of ear tags with common pliers, hair clipping over brands, and blood sampling with individual needles. All cattle were sampled yearly, in the fall, for determination of bovine leukosis virus infection status. All calves born to infected cows were sampled to determine bovine leukosis virus infection status after six mo of age, when colostral antibodies to BLV were no longer present.

Results

Phase I. Results of the initial survey are shown in Table 1. A total of seven cattle in four areas were found to be infected during the initial screen. This sample size was designed to determine the presence of at least one infected animal with 95% confidence if infection rates were at or above 5% in the group. The detection of one or more positive animals gives no estimate of the true prevalence of infection in the population. Thus, further testing was required to determine the prevalence of bovine leukosis virus infection in these areas.

All cattle in the twinner population (areas 52 and 73) were negative. This suggests that the BLV eradication program undertaken following assembly of this herd was successful.

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[1]Published as a research bulletin of the University of Nebraska-Lincoln Agricultural Research Division.

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[3]Appreciation is expressed to the cattle operations staff, W. Gordon Hays, manager, for assistance in collection of samples and to Jill Boyum and David F. Heilman for technical assistance.

[4]The authors acknowledge the contribution of Dr. Dennis Wilson in the design of Phase II of this project.

Further testing of cattle in positive areas and areas where positive cattle may have lived revealed additional positive cattle (Table 2). This included areas 12, 25, 58, 67, 73, and two subpopulations of area 18, the disease resistance and Angus herds. The prevalence rates were 3.9%, .7%, .2%, .5%, .2%, 0%, and 9%, respectively.

Infection status of all dams and progeny of infected cows was determined to assess the possibility of vertical transmission. Infected cows had produced 139 calves. Of seven dams and 48 offspring for whom serum samples were available, two infected dam-daughter pairs were found. In one dam-daughter pair both individuals were infected prior to 1988, thus year of seroconversion could not be determined. In the other pair, the dam seroconverted in 1989 and the daughter seroconverted prior to 1988. Thus, in the first pair the possibility of vertical transmission cannot be ruled out. In the latter pair, vertical transmission is not possible. None of the other 46 offspring showed evidence of vertical transmission.

Of the 30 cows in area 12 found to be infected in 1989, analysis of their 1988 sera showed that six of these cows were negative in 1988, suggesting that active transmission was occurring in area 12.

A summary of the location, population size, number of cattle tested, and number of cattle found infected in 1989 is provided in Figure 1.

Phase II. This project is currently in phase II, yet some preliminary results are available. Blood samples were collected from all positive cows in area 12. Total lymphocyte numbers and T:B lymphocyte ratios were determined to characterize the current status of the animals with respect to bovine leukosis and to help estimate the relative potential of the individual for infectivity to other animals. No evidence of peripheral lymphocytosis or aberrant T:B lymphocyte ratios was detected.

Samples collected from all area 12 cows in the fall of 1990 (n=915) and 1991 (n=953), revealed 8 and 17 newly infected cows, respectively. This further suggests that active transmission is occurring via some means at area 12.

All calves born in 1990 to infected cows (n=32) were sampled in the summer of 1991 and none were found to be infected. Sampling of calves born in 1991 is pending.

Information about the order in which cattle are processed is being collected. This data, combined with determination of the identity of newly infected cows, will be used to evaluate whether or not cattle processed directly after bovine leukosis virus- positive cattle were at greater risk to seroconvert than those processed prior to bovine leukosis virus-positive cattle. This will assess the risk of routine husbandry procedures such as injection with common needles and rectal palpation with common sleeves.

In summary, cows infected with bovine leukosis virus are present in several MARC beef cattle herds, but the prevalence rate is low. The highest concentration of infected cows is in one area at MARC. The management factors that have contributed to this are not known. There is active transmission of bovine leukosis virus in at least one population of cattle. The means of transmission has not been determined. It appears that vertical transmission is not an important contributor.

While the low infection rate present in this population of cattle does not afford the opportunity to evaluate production parameters, it may allow us to identify factors that contribute to field transmission of bovine leukosis virus. The practical implication is that knowledge of how this virus is transmitted allows producers to minimize high risk husbandry techniques, thus reducing the number of newly infected cows.

Table 1—Results of initial screening of adult cattle with a sample size, per area, large enough to detect at least one positive cow if infection rates were 5% or greater. All twinner cattle in areas 52 and 73 were tested.

Area	Number present	Number sampled	Number positive
12	760	56	4
18	754	56	0
25	291	53	1
46	587	56	0
52	361[a]	361	0
53	371	54	0
58	850	56	1
67	950	57	0
73	821[b]	447	1
82	186	50	0
84	150	48	0
99	48	31	0
Total	6,129	1,325	7

[a]Includes 166 adult bulls, all of which were screened, that were not part of the twinner herd.

[b]Includes 430 adult cattle, 56 of which were screened and 1 of which was positive, that were not part of the twinner herd.

Table 2—Results of additional testing of cattle in positive areas and areas where positive cattle may have lived

Group	Number tested	Number positive
Area 12	760	30
Area 25	291	2
Area 58	850	2
Area 67	950	5
Area 73 non-twinner project cows	430	1
Area 18 disease resistance herd	155	0
Area 18 Angus	88	5
Total	3,524	45

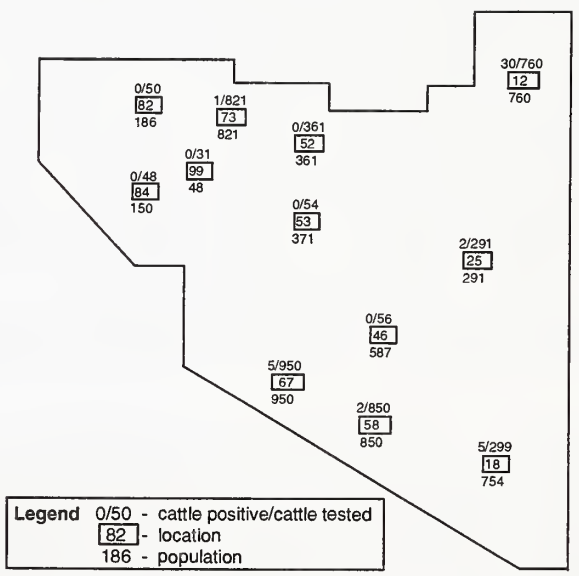

Figure 1 – Summary of the location, population size, number of cattle tested, and number of cattle found infected in 1989.

Isolation of *Pasteurella* spp. from Sick and Healthy Feedlot Calves Using Four Different Sampling Techniques[1]

Keith A. Gilmore, D. Dee Griffin, and Louis J. Perino[2,3]

Introduction

Bovine respiratory disease is the most common disease complex of feedlot cattle. The peak incidence of the disease occurs within the first few weeks of arrival at the feedlot. Bovine respiratory disease is attributed to a complex interaction between bacteria, viruses, environment, stress, and managerial practices. *Pasteurella hemolytica*, and to a lesser extent *Pasteurella multocida*, are considered to be the most common bacterial isolates from cases of bovine respiratory disease.

The purpose of this study was threefold: 1) compare the ability of four different sampling techniques to isolate *Pasteurella* spp. from the respiratory tract of calves, 2) compare the prevalence of *Pasteurella* spp. in the respiratory tract of sick calves and clinically normal cohorts, and 3) evaluate the feasibility and practicality of performing nonsurgical tracheal washes in a feedlot setting.

Procedure

In the fall a group of 64 spring born calves ranging in age from four to six mo were weaned and transported to the MARC feedlot. All of the calves were born and raised at MARC. The calves were vaccinated three wk prior to weaning with a modified live infectious bovine rhinotracheitis virus and bovine viral diarrhea virus (IBR-BVD), polyvalent *Clostridium* spp., and polyvalent *Leptospira* spp. vaccines. Calves were boostered with IBR-BVD and given ivermectin 30 days after weaning and transport to the feedlot. In the feedlot the calves were fed chopped brome hay for the first three days, 40% ground alfalfa hay was added to the diet for the following five days, and silage was introduced after eight days.

The calves were monitored for 28 days in the feedlot. The cattle were observed daily for signs of disease and were removed from the pen when signs of disease became apparent. Calves which exhibited rapid breathing, a runny nose, coughing, inappetence, depression, or isolation were removed to the hospital facility. If a removed calf had a rectal temperature of 103°F or above and the illness could not be referred to any other body system, the calf was treated for respiratory disease. The treatment protocol was intravenous tylosin (8 mg/lb) and oxytetracycline (7 mg/lb) daily for four days, oral sulfadimethoxine boluses (62.5 mg/lb) on the first day and intramuscular vitamin B complex (1 ml/100 lb) on the first day. For each sick calf removed from the pen, sampled, and treated a clinically normal cohort of the same approximate age, gender, and disease history was removed from the pen and sampled but not treated.

Sick and cohort calves were sampled on the day they were removed from the pen before the initiation of treatment and on the last day of the initial respiratory disease treatment. The calves were restrained in the treatment chute and a six inch sterile cotton swab was inserted into the nostril. The swab was then placed into a sterile tube with one milliliter of sterile phosphate buffered saline (PBS). A 13.25 inch guarded nasal swab was then inserted into the nostril. A mouth speculum was used to open the mouth and a sterile 26.25 inch guarded tracheal swab was inserted through the mouth and into the trachea. Both the 13.25 and 26.25 inch guarded swabs were closed systems that contained transport media. Finally, a 36 inch piece of eight millimeter diameter rigid plastic tubing was inserted through the mouth and into the trachea. A sterile length of flexible tubing was threaded down the plastic tube into the trachea. Using a 60 milliliter syringe, approximately 120 milliliters of sterile PBS was injected down the flexible tubing into the distal trachea and quickly aspirated back to obtain a tracheal wash. The total sampling time for each calf was approximately two minutes.

The samples were transported four miles to the Great Plains Veterinary Educational Center Clinical Microbiology Laboratory and plated on blood agar with 5% sheep blood and McConkey II agar. The plates were incubated at 98.6°F in 5% CO_2 and examined at 24 and 48 hr for growth. *Pasteurella* spp. were identified using standardized isolation procedures.

Mantel-Haenszel Chi-squares were calculated using a public-domain microcomputer epidemiologic statistic program (USD Inc, Stone Mountain, Georgia).

Results

Of the 64 calves included in the study, 19 developed clinical bovine respiratory disease within the first 28 days of their arrival at the feedlot. Each of the cohorts remained healthy during the sampling period with the exception of one calf which developed respiratory disease and was then included in the sick group. The morbidity rate was 31% and the mortality rate was 0% for this group of calves during the study period. The results of the *Pasteurella* spp. isolation for each sampling technique are summarized in Table 1.

When the four different sampling techniques were compared on each day for cohort and sick calves there was not a significant difference in the number of *Pasteurella* spp. isolates obtained by any of the techniques.

This information is significant because there is a great difference in the degree of difficulty of sample collection between the four techniques. The performance of all four techniques required minimal time, thus all of the techniques can be performed in the feedlot. The easiest and least expensive of the four is carefully placing a six inch cotton swab into the nasal passage, placing it in PBS, and transporting it to the laboratory. Other techniques involved more elaborate and expensive collection systems.

All of the techniques required handling and restraint of the head of the calf, thus all likely caused some degree of psychological distress. Collection of the two nasal samples required a lesser degree of restraint and manipulation than collection of the two tracheal samples, with the tracheal wash requiring the longest and most vigorous restraint.

Care must be taken with all four techniques to properly restrain the calf. If restraint is inadequate the wooden shaft

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[1]Published as a research bulletin of the University of Nebraska-Lincoln Agricultural Research Division.

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[3]Appreciation is expressed to the staff of the MARC feedlot for assistance in collection of samples and to Jill Boyum and Karen Shuck for technical assistance.

of the six inch nasal swab could break, resulting in loss of the cotton tip in the nasal passage. Epistaxis (nosebleed) could be induced if the guarded deep nasal swab was not introduced with care. The mucosal covering of the lyssa of the tongue could be torn if the tongue was restrained too vigorously during collection of tracheal samples. No other adverse effects that could be attributed to sample collection were noted in any calves.

The main consideration for the selection of a sampling technique is its ability to isolate relevant pathogenic microorganisms. Other important considerations include practicality in a field setting, lack of complications, skill required, ease of sample collection, degree of calf distress induced, and cost of sample collection. These data suggest that when all of these factors are considered, a six inch nasal swab is the most effective sample collection system.

It is not known if isolates were *Pasteurella multocida* or *Pasteurella hemolytica*, as only the genus of isolates was characterized. Additionally, if isolates were *Pasteurella hemolytica*, it is not known if isolates were serotype 1 or 2. This information would allow us to better assess the pathogenic relevance of the *Pasteurella* isolates since pneumonic pasteurellosis in cattle is typically associated with *Pasteurella hemolytica* A1.

Samples were collected after calves were identified as sick. Differences in the microflora between the upper and lower respiratory tract may have been overlooked because the sample was taken after the disease process was well advanced. However, these data suggest that during an outbreak of bovine respiratory disease there is no difference between the sampling techniques examined.

On the first day of treatment there was not a significant difference in the number of calves from which *Pasteurella* spp. was isolated between the sick and cohort groups. Also, there was not a significant difference in the number of calves from which *Pasteurella* spp. was isolated in the cohort calves on day one and day four. For the sick calves there was a significant drop in the number of calves from which *Pasteurella* spp. was isolated from day one to day four of treatment. This is likely a result of antimicrobial therapy.

Differentiation of sick and cohort calves was based on subjective criteria. Since nontreated controls were not included in the sick group, it is possible that healthy calves were erroneously included in the sick group. Misclassification of calves could have affected the outcome of this trial. However, as only one of the cohort calves became sick and none died, misclassification seems less likely.

The treatment protocol used was effective in causing a shift in the microflora of the nasopharynx and trachea. All the calves diagnosed as having bovine respiratory disease and undergoing treatment did recover and remained clinically healthy during the remainder of the study period.

These findings are consistent with and extend previous research. The lower respiratory tract of a normal, unstressed calf is usually sterile. Normal, unstressed calves carry low numbers of *Pasteurella* spp. as part of their nasal flora. Due to changes in the respiratory tract induced by husbandry practices such as weaning and transport, or infection with viruses or mycoplasmas, floral shifts occur that result in an increase in the numbers of pathogenic *Pasteurella* in the respiratory tract. This increase in the challenge dose presented to the defenses of the lower respiratory tract, along with compromise of the defense mechanisms of the lower respiratory tract, results in pneumonic pasteurellosis. Antibiotic therapy does not "cure" the animal. Rather it suppresses bacterial proliferation to such a degree that the pneumonic defenses of the calf can clear the infection.

In summary, there was no significant difference in the ability to isolate *Pasteurella* spp. from sick or cohort calves using either a short nasal swab, a long guarded nasal swab, a guarded tracheal swab, and tracheal lavage. There was no significant difference in the isolation of *Pasteurella* spp. between the sick and cohort calves on the first day of treatment. There was no significant difference in the samples obtained from the cohort calves on day 1 and day 4. There was a significant difference in the ability to isolate *Pasteurella* spp. in the sick calves on day 1 compared to day 4 that is likely a result of antimicrobial therapy.

Table 1—Isolation rate for four sampling techniques used on sick and cohort calves on day found sick and after therapy

Group	Sampling Technique: Nasal swab		Guarded nasal swab		Tracheal swab		Tracheal wash	
Sick								
Day found sick	12/19	(63)[a]	13/19	(68)	10/19	(53)	8/19	(42)
After therapy	2/19	(11)[b]	1/19	(5)[b]	2/19	(11)[b]	1/19	(5)[b]
Cohort								
Day found sick[c]	8/18	(44)	10/18	(56)	9/18	(50)	8/18	(44)
After therapy	9/18	(50)	7/18	(39)	6/18	(33)	5/13	(28)

[a] Data are expressed as number of *Pasteurella* spp. isolates/number of samples (%).

[b] Values differ from other values in column (P<.05).

[c] Cohort calves were removed from their home pen and sampled on the same day as sick calves. They received no treatments. They were held in hospital pens and sampled again the last day of the initial respiratory disease treatment of the sick calves.

☆ U.S. GOVERNMENT PRINTING OFFICE: 1993 775-731

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